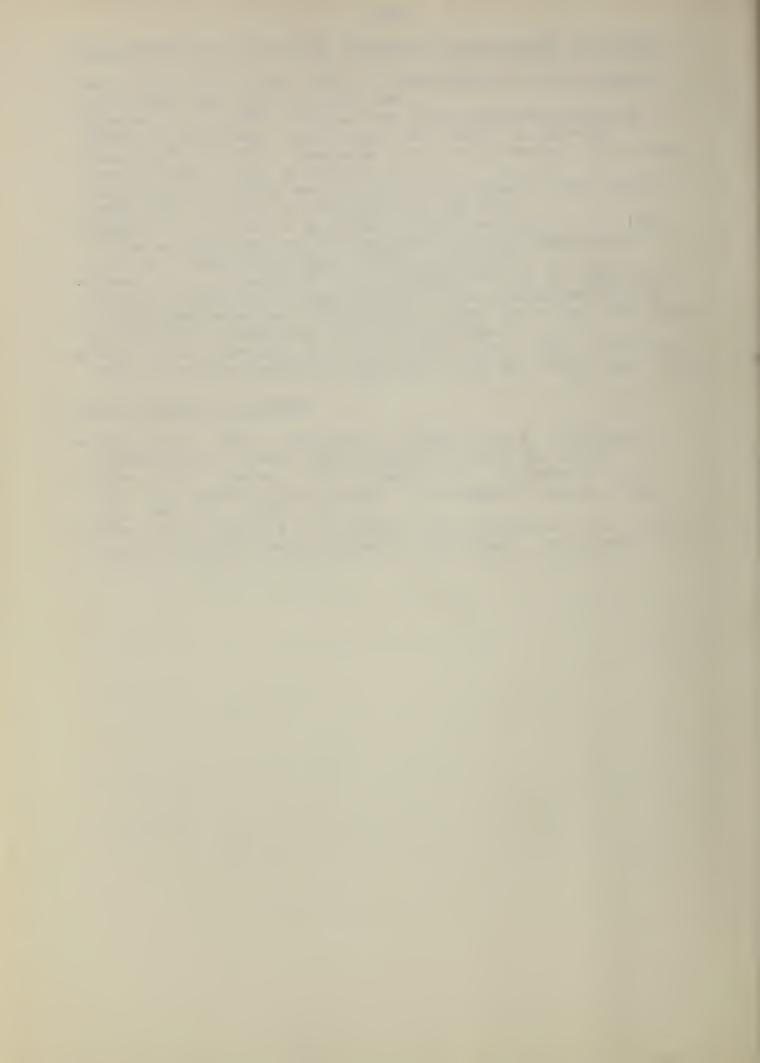
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A Summary of Current Program, 7/1/62

and Preliminary Report of Progress

for 10/1/60 to 6/30/62

ENTOMOLOGY RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

Section B



FEB 23 1960

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This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

There is included under each problem area in the report a brief and very general statement on the nature of the research being conducted by the State Agricultural Experiment Stations and the professional manpower being devoted by the State stations to such research. Also included is a brief description of related work conducted by private organizations. No details on progress of State station or industry research are included except as such work is cooperative with U.S.D.A.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued between October 1, 1960 and June 30, 1962. Current agricultural research findings are also published in the monthly U.S.D.A. publication, Agricultural Research. This progress report was compiled in the Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.

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AREA 14. DAIRY CATTLE INSECTS

Problem. Flies, mosquitoes, grubs, lice, and ticks are common pests of dairy cattle that cause important losses in all parts of the United States. The screw-worm, a serious pest of dairy cattle, is prevalent in the southern and southwestern States. Heavy attacks by biting flies lower milk production by 5 to 20%. Total losses to dairy cattle attributable to insects and ticks are estimated to exceed \$200 million annually. Certain insect pests are also involved in the transmission of diseases of dairy cattle. Methods of control for dairy insects have received setbacks during recent years because the best available insecticides and most promising new materials produce residues in milk. In addition, house flies around dairy establishments have developed resistance to DDT and other insecticides. is, therefore, great need to find safe, effective, nonresidue insecticides and repellents to control these insects and ticks. Effective systemic insecticides and ways of administration which would avoid residues are needed to combat grubs in dairy cattle and to prevent the face fly and horn fly from breeding in the manure. New approaches to control, including radiation and chemosterilants, need to be explored to determine their feasibility for the control of several dairy-cattle pests. Efforts also should be made to find and evaluate insect pathogens, parasites, and predators for controlling certain dairy-cattle pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycle to serve as a basis for the development of more effective and safer methods of control. Research is also urgently needed on the role of insects in the spread of diseases of dairy cattle.

USDA PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of dairy cattle. Studies are conducted on the biology, physiology, genetics and nutrition of the screw-worm fly, stable fly, horn fly, horse and deer flies, the face fly, mosquitoes, and other pests; on the nature of insect resistance to insecticides; on the mode of action of insecticides and on their absorption, metabolism and excretion by insects; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is directed towards the development of more effective conventional and systemic insecticides and protective treatments for the control of dairy-cattle pests. Studies are conducted to determine the occurrence of insecticide residues in the tissues and the milk of treated animals. Minor attention is given to the development of sanitation and management procedures and to biological control,

especially parasites and predators, for controlling the face fly, stable fly, horse fly, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to develop physical and mechanical methods of control, to evaluate traps and devices for estimating and controlling natural insect populations and improved or special equipment for the application of insecticides to dairy cattle. Limited research is conducted on the role of insects and ticks as vectors of animal diseases, with special emphasis on bovine anaplasmosis. The research is conducted in major laboratories at Kerrville, Tex., Corvallis, Oreg., and Orlando, Fla., and at satellite stations at Beltsville, Md., Stone-ville, Miss., Lincoln, Nebr., and Fresno, Calif.

The Federal scientific effort devoted to research in this area totals 15.8 professional man-years. Of this number 5.6 is devoted to basic biology, physiology and nutrition; 3.7 to insecticidal and sanitation control; 2.5 to insecticide residue determinations; 0.3 to biological control; 1.9 to insect sterility, attractants and other new approaches to control; 0.3 to evaluation of equipment for insect detection and control; 0.7 to insect vectors of diseases; and 0.8 to program leadership.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 6.7 professional man-years devoted to insect pests of dairy cattle, divided among subheadings as follows: Basic biology, physiology, and nutrition, 1.0; insecticidal and sanitation control, 3.7; insecticide residues, 1.7; biological control, 0.2; and insect sterility, attractants and other new approaches to control, 0.1.

Industry, especially chemical companies, and other organizations are engaged in research on the formulation and evaluation of insecticides for the control of dairy cattle pests. Industry also cooperates with Federal and State workers in developing information on residues resulting from the use of various insecticides in connection with label registration. Estimated annual expenditures by industry are equivalent to approximately 10 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

1. House Fly. Studies in Oregon showed that DDT-resistance in house flies was attributable to the ability of the flies to dehydrochlorinate the insecticide. The mechanism of resistance in house flies to the carbamates was due to aliesterase activity, the same as for the organophosphates.

Studies in Oregon showed that P³² metepa was rapidly absorbed and metabolized by house flies and mice. Almost complete degradation occurred in 24 hours with phosphoric acid being the major metabolic product.

Studies in Oregon indicated that irradiation of resistant flies with 1000 r did not alter their susceptibility to insecticides or esterase activity. Irradiated females mated with normal males produced normal numbers of eggs but only 45% were fertile.

Further studies were conducted with normal and parathion-resistant flies that had been irradiated as pupae with 600 r for 7 generations. Only about 25% of the eggs hatched from matings of irradiated males and females of either colony. No changes in either esterase activity or insecticide susceptibility were apparent.

In Oregon studies showed that certain synergists greatly increased the toxicity of malathion to resistant strains of house flies. The synergists, simple tris-substituted derivatives of phosphoric acid, completely overcame high levels of resistance when used at 1:1 or higher ratios of synergist to insecticide. The most effective materials increased the toxicity of malathion from 36-to-40-fold against resistant house flies. Only about a 2-fold increase was indicated against susceptible strains of house flies. The relative ability of several of these materials to synergize malathion against resistant house flies was directly related to the inhibitory effect of the synergists on aliesterase activity. Strong synergists for malathion inhibited aliesterase activity at concentrations as low as 10^{-5} M, while related nonsynergistic materials failed to inhibit the aliesterase at concentrations up to 10^{-2} M. The synergists may actually inhibit the mutant aliesterase present in all organophosphate-resistant fly strains.

House flies treated with the synergist tributyl phosphorotrithicate and then treated with either parathion or paraoxon accumulated greater quantities of paraoxon than did flies treated with the toxicants only. This was true with both susceptible and parathion-resistant strains of flies. Measurements of the inhibition of thoracic cholinesterase activity provided a far better picture of the toxic action of organophosphates than did measurements of head cholinesterase.

In Oregon selection of a house fly colony with Isolan produced a strain with 3-fold resistance in 14 generations. At the same time, levels of esterase activity to methyl butyrate declined to 40% of the original level in flies of the selected strain. This same phenomenon occurs when house flies are selected with organophosphates, indicating that the same mechanism is responsible for resistance to both classes of insecticides.

House flies were capable of dispersing a distance of at least 5 miles in 24-48 hours. In one series of tests, males sterilized by feeding 3 days on apholate proved fully competitive with normal males when placed with normal females. When only treated males were placed with fed females all eggs were sterile and 12.5% were sterile when only normal males were present. When fed females, normal males, and treated males were combined at ratios of 1:1:1 and 1:1:2, 65 and 80% of the eggs were sterile and higher ratios of 1:1:3, 1:1:5, and 1:1:10 resulted in 99.9-100% sterility. Additional tests at these ratios confirmed that actual sterility was higher than the expected.

In Florida cytological studies showed that the ovaries in normal 3-day-old flies were 6-8 times as large as those in females fed for 3 days on 1.0% apholate. After feeding ceased some growth occurred but ovaries never attained normal size. Females mated to males fed on 0.4-1.0% apholate oviposited but the eggs showed little or no embryonic development, whereas females mated with males fed 0.1-0.3% laid some viable eggs and some without embryonic development. Similar results were obtained in tests with normal and insecticide resistant strains of flies.

In further studies on the cytological effects 1% apholate given in adult food over a period of 24 hours inhibited but did not eliminate ovarian development in females. The greatest effect was noted at 72 hours after eclosion of the nurse cells in the first and second egg chambers. The chromatin was irregular and nuclei had bizarre shapes. Occytes matured in the first cell but not in the others. The germarium was also affected as the third egg chamber was not visible until 168-192 hours after eclosion compared to 96 hours in normal flies.

2. Mosquitoes. Studies were initiated at Fresno, Calif. in July, 1961, on the biology of mosquitoes in relation to agriculture, especially with regard to irrigation and land management practices, in cooperation with the Soil and Water Conservation Research Division and the Bureau of Vector Control of the California State Department of Health. Farly studies showed that dairy drains are sites of heavy breeding of Culex quinquefasciatus through November and into early December. The breeding in such locations contributes heavily to overwintering adult population of this species. No autogeny (ability to lay eggs without a prior blood meal) was observed in this vicinity with Aedes vexans, Culex apicalis, C. peus, and C. thriambus.

In Oregon studies on flight movements of <u>tarsalis</u> indicated that they move from their resting stations about sunset and return about sunrise. The instinct of <u>tarsalis</u> to oviposit in low sites is stronger than the instinct for oviposition in favorable waters. Female <u>tarsalis</u> mate only once, whereas males mate several times.

Studies were continued in Oregon on insecticide resistance in mosquitoes. Tests showed that DDT-resistant Culex tarsalis larvae breeding on farms and in log ponds were also resistant to closely related compounds, but not to dieldrin or lindane. Further spread of resistance in Oregon Culex mosquitoes was indicated when Culex peus showed some apparent resistance to DDT, and tarsalis, for the first time in the State, showed some resistance to malathion. Studies on the physiology of resistance showed that malathion-resistant larvae of C. tarsalis were more efficient in regulating salt (chloride) uptake than susceptible larvae during exposure to malathion. Resistant and susceptible larvae took up similar amounts of chloride when exposed to 1% sodium chloride alone. Exposure to 1% sodium chloride resulted in an increase in oxygen consumption in susceptible and malathion-resistant larvae, but chloride had no measurable effect on cholinesterase inhibition or accumulation of malaoxon, the principal breakdown product of malathion in mosquitoes. Studies of the insect enzyme systems, cholinesterases and aliesterases, led to the development of synergists for organophosphorus insecticides. Several tris-substituted derivatives of phosphoric acid overcame the resistance of tarsalis larvae to malathion, increasing its toxicity 100-fold to a resistant strain. Only about a 2-fold increase was indicated against normal strains. The ability of the synergists to overcome resistance appears to be related to an increase in the titer of a carboxyesterase enzyme. general, the accumulation of malaoxon was proportionate to the effectiveness of the synergist. Malathion-resistant tarsalis detoxify DDT 2-to-3-times as fast as normal strains, largely through the formation of carboxylic derivatives. The DDT detoxifying enzyme, DDTdehydrochlorinase, in flies does not occur in tarsalis.

Studies in Florida showed that metepa was picked up rapidly by tarsal contact from glass surfaces by both mosquitoes and house flies. House flies and A. quadrimaculatus absorbed approximately the same amount (7 µg./insect) during a 4-hour exposure on surfaces treated at 10 mg./ sq. foot. The quadrimaculatus males were so damaged that they could not inseminate the females but house fly males were normal in this respect and 99% of the eggs produced from matings with virgin untreated females were nonviable. Aedes aegypti showed an average uptake of 2.5 ug./insect, which sterilized but apparently did not impair male activity. Feeding for 3 days on treated food (1% of the chemical in 20% honey solution with mosquitoes, and 0.4% in house fly food) sterilized quadrimaculatus males and caused a high degree of sterility in house fly and aegypti males. At the end of the 3-day feeding period the amount of tagged material, expressed at ug.-equivalents of P32 metera was 3.0, 3.7, and 1.7 in males of quadrimaculatus, house flies, and aegypti, respectively. Exposure of larvae from the third instar through pupation in water treated at 10 p.p.m. failed to sterilize quadrimaculatus or aegypti significantly and the uptake of metepa was low in comparison to the other types of treatments.

Studies in Oregon showed that P³² metepa was rapidly absorbed and metabolized by mosquitoes (C. tarsalis) and mice. Almost complete degradation occurred in 24 hours with phosphoric acid being the major metabolic product.

3. Stable Fly. Studies were continued in Nebraska on the biology of stable flies. Immature stages of the stable fly overwintered in protected situations. None survived in environments subject to frequent freezing and thawing. The first adult flies appeared on June 1 and gradually increased through June. Peak populations occurred during July and August.

Efforts were continued in Nebraska to induce resistance to DDT in stable flies. After selection with topical applications of 0.005-0.075% DDT for 34 generations, no resistance was apparent and the colony was discontinued. However, when 36 p.p.m. of DDT was placed in the larval medium, 3-fold resistance was indicated in 6 generations. After 11 generations (1 year), the colony tolerated 500 p.p.m. on the media and some larvae survived 625 p.p.m. Resistance of about 14-fold was indicated.

4. Face Fly. In Nebraska, adults of the face fly hibernating in homes became active in February. Collected females contained well developed eggs but did not oviposit in the laboratory. Adults were first observed on cattle at Lincoln on May 14 and by late June, herds were lightly infested. High populations persisted from early July until cool weather.

After considerable investigation of nutritional requirements, a thriving colony of the face fly was developed under laboratory conditions. The adult diet consisted of cattle feces, citrated blood, milk, dimalt and bee pollen. Iarvae were reared in cattle feces and pupated in a mixture of sand and vermiculite. The life cycle (egg to egg) was 14 days at 82° F. and 28 days at 70°.

5. Horn Fly. Studies on the biology of the horn fly were continued in Texas and Oregon. In Texas intensive spraying was employed to eradicate horn flies at one location during the late fall, whereas flies persisted in other locations until early December. In the spring flies reappeared at untreated locations nearly 4 weeks earlier than at the treated location. The flies overwintered as diapausing pupae and prepupae. In Oregon horn flies overwintered as pupae in cattle droppings but house flies and stable flies failed to survive. Adult horn flies began emerging from overwintering pupae on May 17 and emergence was complete by June 24. Emergence ranged from 0.8 to 14.8% in exposed droppings and from 3 to 61% in those protected by screen cages.

In laboratory studies in Oregon occasional eggs of the horn fly hatched at a constant temperature of 52° F. and the larvae developed to pupae but no adults emerged from them. Adults also failed to emerge from 1-day old pupae reared at 80° and then kept at a constant temperature of 52° for 3 months but, when returned to 80°, 20% produced adults in 5 days. When 5-day old pupae were transferred from 80° F. to 52° adult emergence occurred between 7 and 23 days, whereas at 80° it was complete in 1 to 3 days.

In Texas a laboratory colony of the horn fly has been developed and maintained for 10 generations without contact with a bovine host. The adult diet consists of bovine blood, ground beef juice and an antibiotic to prevent spoilage. The optimum temperature for survival and reproduction is 90° F. Iarvae are reared in feces of cattle fed alfalfa hay. The feces of cattle fed sorghum or prairie grass proved unsatisfactory as larval media.

6. Screw-worm. Investigations on the screw-worm were conducted at Kerrville, Tex. The sexual development and behavior and genetics of the screw-worm fly were studied. Dissections showed that egg development was synchronous in all ovarioles. The exact stage of development of the oocyte and the nurse cells and the cytological condition of the nucleus were determined for various ages of females. Males began mating when 2-days old but were not vigorous in mating until 4 days of age.

The number of adults in holding cages and mating activity had pronounced effects on longevity. Optimum longevity occurred when not over 100 adults were confined in 12" x 12" x 20" cages. Adults also lived longer when the sexes were kept separate and when the male to female ratio was reduced. Excess mating by males and harassment of females by males reduced longevity.

Efforts were made to develop a genetically distinct strain of screwworm fly. In examining many thousands of adults from scores of strains a black mutant male was discovered. Selected matings resulted in the development of a homozygous black strain. When this strain proved inferior to normal strains in longevity and mating vigor, new genes were introduced by mating black females to normal blue males. After inbreeding for several generations, the black strain was equal to normal strains in longevity and mating vigor. Several additional new strains have been developed from larvae and adults, including one in which the scutellum is fused laterally to the scutum. Linkage studies with "black" and "interrupted" marker strains showed that these genes are sex linked on the "X" chromosome. Crosses of these strains did not produce the expected number of black males, and females homozygous for the "interrupted" gene did not survive as well as the heterozygotes.

Efforts to formulate a completely synthetic larval rearing medium have proved unsuccessful so far, but progress has been made in developing

a modification of the standard ground meat-blood medium. Screw-worm larvae reared for 48 hours on the standard medium consisting of 50% horse meat, 30% bovine plasma and 20% water were successfully transferred and completed development in media in which 50, 75, 87.5, and 100% of the horse meat had been replaced with fish flour. The substitution of the fish flour reduced production costs to as low as 8.7¢ per thousand pupae, compared with 21.4¢ for the standard medium. Weights of the larvae ranged from 73.0 to 69.6 mg. for the 50 to 87.5% substitutions, as compared with 74.6 mg. for the standard. The 100% substitutions yielded larvae that weighed only 60.2 mg., but the adults lived slightly longer than those produced on the standard medium. In cage tests the small males and normal-sized males mated about equally as well with normal-sized females.

Intensive studies were made on the effects of irradiation on the sexual development, mating ability and longevity of the screw-worm. A dosage of 5,000 r completely sterilized both sexes. No oviposition resulted from matings of irradiated males and females and normal males and irradiated females, whereas matings of irradiated males and normal females resulted in normal oviposition but none of the eggs hatched. Female flies 0-.4 hours old were more sensitive to irradiation than 5-day old pupae. A dosage of 2,000 r reduced ovarian growth by 50% in young females but had no significant effect on ovarian growth in females irradiated as 5-day old pupae. Irradiation had little or no effect on ovarian growth in females over 48 hours old. However, oocytes in 4 to 5-day old females were damaged more by irradiation than those in 3-day old flies. No difference was noted in the longevity of 5-, 6-, and 7-day old flies irradiated with 7,500 r in one, two, or three exposures. The number of dominant lethals and longevity of adults were unchanged regardless of whether 5-day old pupae were irradiated with a single dose or equal fractions at intervals of 8 and 24 hours.

There was no consistent difference in the effects of 1,000 and 5,000 r administered as a single dose or two equal doses. Female flies 3-, 4-, and 5-days old differed greatly in radiation sensitivity but dominant lethals were induced in the oocytes of all ages. In additional tests with 5-day old pupae, which have only oogonial cells, the number of eggs deposited by females decreased as the radiation dose increased, indicating that damage to some cells eliminated them from the germ line and that they were not rapidly replaced.

Additional studies on the effects of irradiation on 4-, 5-, 6-, and 7-day old screw-worm pupae showed decreased longevity of adults with increased dosage. All stages showed lower mortality at 2800 r than at higher dosages.

When female screw-worms were irradiated as 5-day old pupae in a well-aerated container, a dose of 5000 r produced 99% sterility. When the

same dose was given in an atmosphere of carbon dioxide less than 50% sterility resulted. However, irradiation in an atmosphere of 50% air and 50% CO2 produced a greater effect than in air alone. These results suggest that the irradiation capacity of the present Cobalt-60 sources could be increased by 38% by irradiating with 5000 r in 50% air-50% CO2. For this synergistic effect to be shown, it is necessary that the insects be held in the CO2-air mixture for at least 30 minutes prior to, as well as during irradiation. It should be a relatively simple engineering job to modify the currently used procedures for such irradiation.

Studies were conducted on the absorption, distribution, metabolism, and excretion of P³² metepa (chemosterilant). Metepa was absorbed faster and excreted more slowly by the stable fly than the screwworm fly. This may account for the fact that the sterilizing dose of metepa is much less for the stable fly than for the screw-worm fly.

Field cage studies in Texas showed that mature screw-worm larvae released on soil at 73° F. entered the ground in 3-4 minutes and pupated in 24 hours. Peak emergence of adults occurred 12 days later between 5:00 and 8:00 a.m. Adults did not feed or exhibit mating activity until 4 days old. Adult survival was high for 9 days, but very few adults were alive after 22 days. In similar releases when soil temperatures were 51° F. few larvae entered the soil and although many were alive after 1 week, none had pupated.

Work was conducted on the dispersal and behavior of marked released adult screw-worm flies. The first night after release from 0.4 to 10% of the marked flies were recovered within 25 yards of the release point. Recoveries decreased on successive nights. In some tests none apparently moved over 400 yards, whereas in others, dispersion was rapid, as far as 1 mile in 45 minutes. Over 90% of the adults were found resting near the tips of leafless twigs within 4-5 feet of the ground.

- 7. Cattle Grubs. Research was initiated in Texas and Oregon to determine the nutritional requirements of cattle grub larvae and to develop an artificial medium for rearing larvae under laboratory conditions. Second and third stage larvae have been successfully reared to maturity in serum in shell vials, but adult emergence from the pupae was quite low. Efforts to develop a suitable medium and techniques for rearing first stage larvae have proved unsuccessful so far.
- 8. Horse Flies and Deer Flies. Studies were continued in Mississippi on the biology of tabanids. The earliest species, Tabanus lasiophthalmus, appeared on March 31, reached a peak by late April, and disappeared by late May. T. vittiger schwardti, the most prevalent species, appeared April 5, was abundant from mid-May until

early July and small numbers persisted until cool weather. T.
fuscicostatus appeared May 11, reached a peak in June and disappeared
by early September. Other species of lesser importance and dates of
their occurrence were as follows: T. proximus, June 8 to September 7;
T. lineola, April 26 to October 5; T. americanus, May 24 to August 17;
and Chrysops flavida, May 11 to October 19. Eighteen species were
collected during the season. Several species of adult flies were
successfully collected and fed in confinement on cattle. Females of
T. proximus and T. vittiger schwardti produced egg masses in captivity
and the eggs hatched in 7 and 4 days, respectively.

- 9. <u>Lice</u>. Studies were conducted in Oregon to determine the factors involved in the decrease of cattle louse infestations during the spring and summer and the increase of populations during the fall and winter. The free cattle harbored very few lice after shedding their winter coat in the spring, but when they were restrained heavy infestations developed within a month. When released the cattle licked and rubbed themselves constantly for several days, causing a precipitious decline in louse populations. During the winter the heavy coats on the animals apparently minimized the effects of rubbing and licking and as a result high louse populations persisted throughout the winter.
- 10. Ticks. Work in Texas indicated that irradiation at the rate of 1000 r had no effect on the molting of unfed or engorged nymphal lone star ticks. However, a dose of 2500 r prevented molting of unfed and 1-day engorged ticks and only 3% of those engorged 1 week molted to adults. Exposure of newly molted nymphs to 1000 r completely sterilized the males but not the females.

B. Insecticidal and Sanitation Control

1. House Fly. Investigations on these insects were conducted at Orlando, Fla. One hundred ninety-five new compounds were screened for residual effectiveness against house flies. Twenty-four materials were 90-100% effective for at least 24 weeks as compared with 12-16 weeks for malathion (standard). These materials included Thiodan, General Chemical GC-3583, Shell SD-4402, Upjohn TUC U-12927 (with synergist); Bayer compounds 29952, 30237, 30468 and 29492; Hooker HRS-1422, Geigy G-27365, Stauffer N-2404, heptachlor epoxide and twelve confidential compounds. Bayer 29952, Bayer 30237 and seven of the confidential compounds were still 100% effective after 44-48 weeks and are considered unusually promising for controlling flies. Several promising new compounds were evaluated as space sprays against susceptible and resistant strains of house flies. Some of the compounds were superior to malathion (standard) against susceptible flies but only one, Bayer 30911, was superior against resistant strains.

Over 50 selected compounds were evaluated as bait toxicants against resistant and normal strains of house flies. Eight of the compounds were equal or superior to Dipterex (standard) against both strains of flies, namely, Bayer 30237, Bayer 30554, General Chemical 6506, Stauffer N-2230, Stauffer N-2404, dimethoate and two confidential compounds. All of the compounds tested were more effective against normal than against resistant flies.

Several materials which increased the effectiveness of malathion in laboratory tests were evaluated as residual treatments in barns against large populations of flies. Combinations of several synergists and malathion alone were equally ineffective in these tests.

2. Mosquitoes. Studies were continued in Florida to find more effective residual insecticides for controlling mosquitoes. Of over 500 new compounds screened for toxicity to Anopheles quadrimaculatus larvae, five were outstanding in their effectiveness. Of 157 selected compounds screened against female adults of Anopheles quadrimaculatus, 17 were very promising causing 90 to 100% kill of exposed quadrimaculatus for 24 weeks. Four of the compounds, Bayer 39007, Hercules 7522C, Bayer 34098, and Bayer 4183, retained their effectiveness on sprayed surfaces for 1 year. In tests with treatments that had aged for 2 years, Sevin and Hercules AC-5727 plus piperonyl butoxide caused 85% kills of quadrimaculatus within 24 hours after a 1-hour exposure to 100 mg./sq. ft., whereas malathion and unsynergized AC-5727 were ineffective.

In Florida, tests in the laboratory and field were continued to develop more effective materials for mosquito control. Of 151 selected compounds tested as space sprays against colonized Aedes taeniorhynchus, four were better than the malathion standard: Kenya Oleo Resin, Bayer 34042, Bayer 30749 and Hercules 7522C. In aerial spray tests against natural populations of salt-marsh mosquito adults, 0.05 pound per acre of Dibrom or DDVP was about as effective as 0.1 pound of malathion per acre. The sprays reduced mosquito abundance more than 90% in 6 hours.

In Oregon screening tests were conducted with a number of new insecticides against larvae and adults of <u>Oulex tarsalis</u>. Three of the materials gave 100% kills of larvae at a concentration of only 0.025 p.p.m. In spray tests seven materials were 2 to 9 times more toxic to adult mosquitoes than malathion (standard) and one, Bayer 39007, compared favorably with parathion.

In Oregon studies were continued to find repellants for protecting animals from mosquitoes. None of 200 compounds screened in spot tests on cattle showed promise.

3. Stable Fly. In Texas, where the research on the stable fly was done, 447 compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Of these materials 9 were Class IV repellents at 5%, 8 were Class IV repellents at 10% and 3 were Class IV toxicants at 0.5% and 2 were Class IV toxicants at 5%. The outstanding repellents were diamyl tartrate, 3-ethyl-2,4-octanediol, benzyl acetoacetaxc, piperonyl chrysanthemumate and Hercules AC-6432. Three other materials were slightly less effective. The most effective toxicants were Geigy G-27365 and Shell SD-2359.

Comparisons were made of the toxicities of various insecticides to stable flies. The LD-50's in ug/fly for the materials tested were as follows: Ronnel, 0.015; methoxychlor, 0.057; DDT, 0.072; DDT and WARF, 0.091; toxaphene, 0.17; Diazinon 0.021; pyrethrins, 0.021-0.028; and pyrethrins and piperonyl butoxide, 0.017.

Large cage tests were conducted on calves to compare the residual effectiveness of a number of new insecticides and methoxychlor (standard). Ruelene, Baytex, Bayer 22408 and Sevin were effective for 3 to 5 days against stable flies but were less effective than methoxychlor.

Extensive tests with pyrethrum showed that ultra-violet light was the most important factor in its rapid loss of effectiveness, although infrared was also deleterious. Wave lengths in the range of 2600-4000 A caused the greatest loss in effectiveness of pyrethrum. Several hydroquinone antioxidants and lanolin prolonged the toxicity of pyrethrum about two-fold under irradiation. Several phosphoric acid derivatives increased the effectiveness of malathion against stable flies but had no effect on Sevin or methoxychlor.

Of 111 compounds tested as systemics, only 8 caused mortality of stable flies feeding on guinea pigs. Hercules 7522-H and Rhodia RP-9895 were effective orally at 50 and 100 mg./kg. The other six materials were effective orally at doses of 10 to 50 mg./kg. but their chemical names are confidential. Thirty Class IV repellents were administered orally to guinea pigs to determine if they would prevent feeding by stable flies. All were ineffective.

4. Face Fly. In Nebraska a number of insecticides were administered to cattle in feed in order to determine levels necessary to prevent fly breeding in the feces. Daily doses of V-C 13 at 4 mg./kg., Cygont at 5 mg./kg. and Co-ral at 10 mg./kg. gave 100% control of breeding but seven other materials were only partially effective at the doses tested. Sprays of methoxychlor, malathion, Delnav and synergized pyrethrins provided little or no control of this pest. Halters treated with DDVP, Dipterex and methoxychlor reduced fly populations but did not provide satisfactory control. Effective control of breeding was obtained by feeding Co-ral at 0.5 mg./kg. and ronnel at 7.8 mg./kg. daily but adult populations on the cattle remained high, presumably because of migration

from adjacent farms. Weekly applications of 0.08 lb./acre of Co-ral on pastures prevented fly breeding in droppings of cattle but here again no effect on adult populations was apparent.

Horn Fly. In Texas tests were conducted on calves in large cages 5. to compare the residual effectiveness of a number of new insecticides and methoxychlor (standard) Ruelene, Baytex, Bayer 22408, and Sevin were effective for 7-10 days as compared with 17 days for methoxychlor. Extensive field tests were conducted in Texas and Mississippi to compare several old and promising new insecticides for the control of horn flies on cattle. In Texas effective control was obtained with 0.25% sprays of GC-4072 for 15-21 days, with 0.06% Diazinon for 8-12 days, and with 0.5% Dilan, Dipterex and methoxychlor for about 2 weeks. In Mississippi control was obtained for 11-14 days with 0.1-0.25% sprays of ENT-27717, for 7-10 days with 0.25% Sevin, and 0.1% Baytex, for 3 weeks with 0.5% Dilan and methoxychlor, for 10-30 days with 0.25-0.5% Shell 4294. In season-long tests, effective control of horn flies was maintained with 4-5 sprayings with 0.25% Shell 4294, with seven sprayings with 0.1%, and with two applications of 2% in oil to backrubbers. Effective control was also maintained with mist sprays of 1% Shell 4294 applied weekly and of 0.05% pyrethrins and 0.01% DDVP applied daily.

Extensive tests were conducted to determine the effectiveness of low level feeding of insecticides in controlling horn flies. In Texas daily dosages of 7.8 mg./kg. of ronnel prevented horn fly breeding in droppings and reduced adult populations to a sub-annoying level within a week. Free choice feeding by cattle on ronnel salt blocks also provided very good control. Other effective materials and dosages were as follows: Co-ral, 0.5 mg./kg.; Bayer 22408, 1 mg.; GC-4072, Baytex, Bayer-34727 and Stauffer R-1504, 2.5 mg./kg.; and Bayer 37342, American Cyanamid 38023 and Bayer 37341, 10 mg./kg. In Mississippi free-choice feeding on ronnel salt blocks and daily feeding at 4 mg./kg. prevented breeding in droppings and adult fly populations decreased significantly.

6. Screw-worm. In Texas research was continued to develop more effective insecticides for controlling screw-worms affecting livestock. One hundred thirty-one new compounds were screened for systemic action by administering them orally and subcutaneously (SC) at several dosages to guinea pigs artifically infested with screw-worms. Ten of the materials showed systemic action in one or both types of administration. The active compounds and minimum effective dosages in mg./kg. were as follows: Stauffer N-2310, Stauffer N-2599 and Stauffer N-3055, 25 mg. orally and 50 mg. SC; Stauffer N-3054, 10 mg. orally and 50 mg. SC; Hercules 9699, 50 mg. orally and SC; Stauffer R-3422, 50 mg. orally and 100 mg. SC; Rhodia 9895, 50 mg. orally and Hercules 7522H, 100 mg. orally.

Screw-worm larvae surviving the screening tests of new compounds were collected and reared to adults. The adults were mated and records made of the number of eggs and percent hatch to determine if the chemicals screened produced any sterilizing effects. Of 121 compounds used in this study 13 adversely affected survival and development of larvae and pupae or oviposition and egg hatch. The most active materials and their effects were as follows: Bayer 38636 at 5 mg. orally, all male survivors; Shell 7079 at 50 mg. orally, no oviposition; Stauffer R-2404 at 10 mg. orally and SC, no hatch; ENT 5734 at 100 mg. SC, adults emerged but died without ovipositing; and Dilan at 500 mg. orally, no adult emergence from pupae.

7. Cattle Grubs and Other Bots. Research was continued in Texas, Oregon, and Nebraska to develop more effective insecticides for controlling cattle grubs and other bots affecting livestock. The ten out of the 131 materials screened in research on the screw-worm in Texas showed systemic action and are promising for further work in the control of cattle grubs.

In Texas further tests were conducted on small numbers of cattle with a number of compounds that had shown promise in screening tests or on individual cattle in 1960 and 1961 and with older effective materials administered in different ways. Ten of the new materials gave 98-100% control of grubs by one or more routes of administration. These materials, the effective doses, and routes of administration were as follows: Famophos, 25 mg./kg. intramuscularly (IM) and 10 mg./kg. in feed for 10 days; Bayer 37341, 0.5% spray, 2% pour-on, 10 mg./kg. orally, and 5 mg./kg. in feed for 10 days; Bayer 34727, 0.5% spray and 25 mg./kg. orally; Stauffer 3352, 25 mg./kg. orally; Stauffer 3828, 100 mg./kg. orally; Stauffer R-1504, 0.5% spray, 2% pour-on, 50 mg./kg. orally and 25 mg./kg. in feed for 10 days; Bayer 37342, 0.5% spray, 25 mg./kg. orally, 15 mg./kg. IM, 5 mg./kg. in feed for 10 days and 10 mg./kg. in feed for 6 days; Bayer 37289, 25 mg./kg. orally; Bayer 42600, 25 mg./kg. orally; and Rhodia, 100 mg./kg. orally.

The older materials, dosages and routes of administration giving 98-100% control of grubs were as follows: Co-ral, 2% and 8% pour-on; Dipterex, 5 mg./kg. in feed for 10 days, 6% pour-on and 150 mg. orally; Baytex, 2.5 mg./kg. in feed for 10 days and 10 mg. IM; Butonate, 5 mg. in feed for 5 days and 10 mg. in feed for 10 days; and Ruelene, 10 mg. IM and SC. In additional tests with GC 4072 one and two sprays of 0.25% gave 94% control. Several other materials were 78-91% effective by one or more methods of administration.

Extensive field tests with government and cooperator herds of cattle were conducted in Texas, Oregon and Nebraska to evaluate the effectiveness of promising new and several older systemics at different rates and various methods of administration. In Texas 99-100% control of grubs was obtained with Co-ral at 5 mg./kg. IM and as a 4% oil solution applied to the backline by the pour-on method. Pour-on

applications of 4% in oil and 2% in oil and water and 0.5% sprays were over 90% effective. Dipterex applied as a 1.5% spray and at 4% and 7.75% pour-on treatments gave 99-100% control of grubs. Ten percent pour-on applications of ronnel were 99% effective. Ruelene gave 95-100% control when applied at 2%, 4% and 7.75% by the pour-on method and as a 0.5% spray or dip. Baytex as a 1% pour-on and as a 0.25% spray gave 96 and 98% control. New materials giving 94 to 99% control were Bayer 37342 as a 0.5% spray and Famophos at 15 mg./kg. IM. Some of the older and newer materials failed to provide satisfactory control at lower dosages or by other methods of administration.

In Texas tests involving a number of cooperator herds were conducted to evaluate ronnel in salt blocks and as a feed supplement for the control of grubs. Feeding ronnel at the rate of 7.8 mg. daily for 14 days failed to give satisfactory control. Consumption of 2.6-2.8 mg./kg. daily of ronnel from the salt blocks for 4 months gave 78 to 86% control of grubs. Excellent control of 97% was indicated in herds given 7.8 mg./kg. of ronnel for 14 days and then provided with ronnel salt blocks for 3 months.

In Nebraska field tests were run to evaluate the effectiveness of Famophos as an intramuscular (IM) injection against cattle grubs. Injections of 15 mg./kg. gave 99% control as compared with 90% for 7.5 mg./kg.

In tests in Oregon with older materials 98 to 100% control of grubs was obtained with the following treatments: Ruelene as 0.25 and 0.5% sprays; Dipterex as 1% and 2% sprays; Co-ral as a 0.5% spray; Baytex as a 0.25% spray, and a 25 mg./kg. in oil pour-on treatment, and in 5-day feeding tests at 5 mg./kg. Baytex also gave good but variable control at lower rates as sprays and pour-on treatments. In tests against grubs already present in the backs of cattle, 85-100 % kill was obtained with pour-on treatments of Baytex at 25 mg./kg. and with 2.5% dusts of DDVP and Dibrom. In tests with new materials excellent control was obtained with Bayer 37342 as a 0.25% spray and orally at 50 mg./kg. and with Famophos as a pour-on at 15 mg./kg. and in feed at 10 mg./kg. for 5 days. Bayer 37341 was highly effective as a 0.25% spray in some tests but not in others. Lower dosages of these materials and all dosages of several other new materials failed to give satisfactory control of grubs. Feeding of ronnel at the rate of 7.8 mg./kg. daily for 14 days resulted in reductions of 94 to 98% in grubs in several groups of cattle.

In Oregon topical application tests showed that male H. bovis adults were more tolerant of ronnel than females. The LD-100's were 180 and 100 ug/fly, respectively.

8. Horse Flies and Deer Flies. In Mississippi studies showed that daily applications of synergized pyrethrins with an automatic sprayer

greatly reduced the number of horse flies and deer flies attacking cattle. Sprays of insecticides, such as toxaphene, malathion and Ciodrin, were ineffective in repelling biting flies but a good percentage of those that engorged died subsequently from contact with the insecticide.

9. <u>Lice.</u> In Mississippi 25 promising new insecticides were evaluated in spot tests in comparison with methoxychlor against cattle lice. Two materials - General Chemical and Bayer 37342 - prevented reinfestation for 12 days as compared with 7 days for methoxychlor. Heavy infestations of horse lice were eradicated with 0.5% malathion sprays. Nine materials were evaluated for systemic action by giving them orally to louse-infested cattle. Co-ral caused 100% kill of all motile lice but the other materials were partially or completely ineffective at the dosages tested.

In Texas extensive field tests were run to compare the effectiveness of several of the newer insecticides against cattle lice. Sprays of 0.25% GC-4072 eradicated lice in a majority of tests but in others, light infestations developed within 2-4 weeks. Ronnel and Shell 4294 at 0.25% gave 100% immediate control but light reinfestations were apparent in all locations in 2-4 weeks. Ruelene applied at the rate of 75 mg./kg. along the "back line" eliminated louse infestations but lower dosages of 25 and 50 mg./kg. were not completely effective. Feeding of 50-75 mg./kg. of Ruelene over a period of 3 days reduced louse infestations only 40-50%. Daily feeding of Co-ral at 0.25 mg./kg. and Bayer 22408 at 0.5 mg./kg. for 3 weeks or more had no noticeable effect on louse infestations.

In Oregon complete control of cattle lice was obtained with sprays of 0.5% Co-ral, 0.25% Baytex and 0.75% lindane. These treatments gave complete control of cattle scabies, (Chorioptes bovis).

10. <u>Ticks</u>. Work on ticks was confined to the Texas laboratory. Only 4 of 131 compounds screened for systemic effectiveness showed systemic action against ticks engorging on treated guinea pigs. The effective materials, dosages (mg./kg.) and routes of administration were as follows: Rhodia, 25 mg. orally and 50 SC; Hercules 9699, 50 mg. 0 and SC; Stauffer N-2310, 50 SC; and Zectran, 50 mg. orally.

Evaluation was made of the effectiveness of 71 insecticides against Boophilus ticks, using the dipping technique. A majority of the materials were toxic to this species, but the most effective were lindane, Co-ral, Ceneral Chemical 3582, Ceneral Chemical 4072, Bayer 25141, Bayer 29952, Bayer 30237, and Bayer 37341.

Extensive field tests were conducted to compare the effectiveness of several insecticides against the winter, lone star, and black-legged ticks on cattle. Complete control of existing infestations of the

winter and black-legged tick was obtained with sprays of 0.1% General Chemical 3582, 0.25% Shell SD-4294 and General Chemical 4072, 0.5% methyl Baytex and toxaphene. Dilan at 0.5% and V-C 13 at 0.25% eliminated the black-legged but not the winter tick. Sprays of 0.05% Diazinon and 0.5% V-C 13 gave lower immediate kills of the winter tick than 0.5% toxaphene but all three treatments were 99-100% effective after 1 week. In tests on horses, 0.25% sprays of GC-4072 and Dilan, and 0.5% sprays of toxaphene gave 100% immediate control of winter and black-legged ticks but only GC-4072 prevented reinfestation within 1 month.

Field tests indicated that 0.5% toxaphene sprays were slightly more effective against the lone star tick on cattle than any of the newer insecticides. However, excellent immediate control of this species was obtained with sprays of 0.025 and 0.05% Diazinon and 0.5% Dilan and V-C 13. In all cases light to moderate reinfestations occurred in 1-2 weeks. Feeding of ronnel at 7.8 mg./kg. daily for 14 days caused no reduction in tick populations on cattle.

In comparative tests 0.5% toxaphene emulsion applied with a Bean sprayer gave better control of winter ticks on cattle than when applied with a Spray-Foil machine. However, only 1 pint of emulsion was applied with the Spray-Foil as compared with about 1 1/2 gallons with the Bean sprayer.

C. Insecticide Residue Determinations

1. <u>Residue Studies</u>. Research was conducted in Texas and Maryland on the absorption, distribution, storage and metabolic fate of insecticides in animals, using chemical and radiometric methods of analysis.

In Texas a steer given 1.88 mg./kg. orally of Cl4 Phosphamidon showed peak activity in the blood in 12-18 hours, in the urine at 4 hours, and in the feces at 24 hours. Little or no activity was detectible after 144 hours. Of the dose received, 71% and 5% was excreted in the urine and feces, respectively. At least 6 metabolites were found but none could be identified. Radiometric analyses did not show significant residues in muscle or fat although the method was sensitive to 1.0 part per billion.

Special tests were conducted in Texas to compare the metabolism of P^{32} Dipterex in two cows which had shown widely variable results in tests with systemics against cattle grubs. Peak activity in the blood and urine of the two animals occurred at the same time but was twice as high in one as in the other. Over a 3-day period one animal excreted nearly twice as much activity in the urine as the other. A comparison of the metabolites indicated that one animal could destroy the less polar compounds much faster than the other. These differences in

the metabolism of Dipterex indicate that the effectiveness of systemics against cattle grubs may be correlated with the animals ability to metabolize the insecticide.

In Texas analytical methods were developed for determining the amounts of ronnel and V-Cl3 in tissues of animals that had been treated with these insecticides. The method was based on the alkaline hydrolysis of the compounds and the determination of the corresponding phenols with amino antipyrene.

In Maryland dairy cattle grazed on pastures treated with as little as 0.25 lb./acre of chlordane still showed small amounts of heptachlor in their milk 8 weeks after treatment. Additional feeding studies with dairy cattle were conducted with corn ensilage containing 1.2, 2.5 and 5.6 p.p.m. of dimethoate. These levels of dimethoate produced only trace amounts in the milk. Fourteen days after being placed on a dimethoate-free diet, no residues could be detected in the milk. In a study with ensilage containing Diazinon no residues could be detected in the milk of cows consuming as much as 500 p.p.m. of Diazinon per week.

2. Toxicity Studies. Studies were conducted in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity to livestock of insecticides and other materials applied by different routes of administration. summary of the results are presented. Detailed results will be given under Unit 2, Animal Diseases and Parasites (ADP a7-11 and ADP a7-12). Extensive tests were run to determine the toxicity of a number of insecticides administered orally, dermally, and by intramuscular injection to cattle. In conventional spray tests with cattle, three of seven insecticides caused no symptoms of toxicity. These materials and the concentration used were Bayer 34727 at 0.25%; Stauffer R-1504 at 0.5% and Rhodia 9895 at 2.0%. One of three animals was affected by a 0.5% application of Bayer 39193. In pour-on tests Bayer 37342 at 2.0% (125 ml.), Bayer 37342 and Bayer 34727 at 2.0% (250 ml.) and GC-4072 at 1.0% in oil (250 ml.) produced no symptoms of toxicity. GC-4072 at 1.0% in water, Bayer 37341 and Stauffer R-1504 at 2.0%, affected some animals and not others. When given orally in capsules to cattle, the following materials were nontoxic: Bayer 37341 and Bayer 37289 at 10 mg./kg., Hercules 9699 at 13.4 mg., Stauffer N-3047 at 15 mg., Bayer 39193 and Stauffer N-2310 at 50 mg., and Neguvon and Rhodia 9895 at 100 mg./kg. Several other materials were toxic to some animals at the dosage tested. In similar tests with calves, 4 of 7 materials were nontoxic at low test dosages but toxic at higher dosages. No toxic symptoms were evident in cattle given 10 daily dosages of the following compounds in feed: V-C 13 at 3 mg./kg., Neguvon at 5 mg.; GC-4072, Baytex and Stauffer R-1504 at 2.5 mg., and Butonate 20852 and Famophos 25644 at 10 mg./kg. Butonate, Bayer 37341, Bayer 34727, and Rhodia were toxic to some animals. Special tests were run

to determine the effects of Dibrom mist sprays as applied to the heads of cattle in controlling the face fly. Repeated applications of approximately 64 cc. of 1.25% and 24 cc. of 1.04% Dibrom produced ocular discharges in most of the animals and opaque spots on the eyes of several.

During the year a number of promising insecticides were applied at high concentrations (5-10%) with a chromatography sprayer at the rate of 100 ml. per animal. Seven compounds caused no obvious toxic symptoms but several lowered the cholinesterase (ChE) of treated animals.

D. Biological Control

In Nebraska large scale releases of the pupal parasites were initiated early in June and continued until early fall at the stockyards in Omaha and in a 36 sq. mile farm area near Lincoln in an effort to control stable flies. The percent parasitism was low during the first month of releases and varied greatly (0 to 100%) from week to week but the average level of parasitism apparently was sufficient to keep populations at a subannoying level throughout most of the season. The parasite Spalangia muscidarum was most effective in dense, compact breeding habitats, whereas Muscidifurax raptor was most effective near the surface of breeding sites.

E. Insect Sterility, Attractants, and Other New Approaches to Control

1. House Fly. Studies in Oregon indicated that irradiation of resistant house flies with sub-sterilizing doses of 1000 r did not alter their susceptibility to insecticides or esterase activity. Treated females mated with untreated males oviposited normally but only 45% of the eggs hatched. Additional studies were conducted with normal and parathion-resistant flies that had been irradiated as pupae with 600 r for 7 generations. Only about 25% of the eggs hatched from matings of irradiated males and females of either colony. No changes in insecticide susceptibility or esterase activity were apparent.

Extensive research on sterilization, attractants and other new approaches to control of the house fly was conducted at Orlando, Fla., in connection with the control of the house fly in dairy barns.

Irradiation caused greater damage to early (2-4 days old) house fly pupae than to middle-age pupae. Virtually no damage was apparent in old pupae and longevity of adults was greater than that of younger pupae. Irradiation of pupae 0-36 hours before adult emergence did not produce complete sterility and some recovery was indicated since second matings resulted in a slight increase in progeny. No recovery occurred in pupae irradiated 72-96 hours before adult emergence.

Extensive studies were conducted to find materials that will induce sterility or otherwise affect the growth and development of the house fly. Of about 1300 compounds tested in the adult food, 21 caused sterility in flies. Only one of about 800 materials tested in the larval media caused sterility but nearly one—third were toxic to the larvae. In secondary tests with 59 compounds that had shown promise in screening tests, twenty—seven caused sterility (no oviposition or hatch) at concentrations of 1% or lower. Additional tests were run with 50 promising materials applied as larval dips, topically to adults, and in the adult food. None caused sterility as larval dips but in adult food, six induced complete or nearly complete sterility. Two other materials were effective only as topical applications.

Special tests were run with 24 promising chemosterilants to ascertain the effects on each sex. Only one material, an aziridinyl compound, caused sterility in both sexes. Methiotepa, 5-fluoroacetic acid, metepa and a confidential material caused complete sterility in males but not always in females. Three materials - 5-fluoroacetic acid, an aziridinyl compound, and 5-fluorouracil - were effective when fed to both sexes.

Studies were conducted to learn more about the action of several effective chemosterilants on house fly sexual development, mating and reproduction. In one series of tests, males sterilized by feeding 3 days on apholate proved fully competitive with normal males when placed with normal females. When only treated males were placed with normal females all eggs were sterile and 12.5% were sterile when only normal males were present. When normal females, normal males, and treated males were combined at ratios of 1:1:1 and 1:1:2, 65 and 80% of the eggs were sterile and higher ratios of 1:1:3, 1:1:5, and 1:1:10 resulted in 99.9-100% sterility. Additional tests at these ratios confirmed that actual sterility was higher than the expected. Males given food containing 0.4-1.0% apholate for 3 days were sterilized for life but lower concentrations of 0.1-0.3% were not 100% effective. posures of males on residues of 500-1000 mg./kg. of tepa on plywood panels caused only partial sterility (12-72%) but when applied at 250-500 mg./sq. ft. with sugar tepa and apholate produced 91-100 and 99-100% sterility, respectively. When applied in glass jars, residues of 250 mg./sq. ft. of tepa or metepa completely sterilized flies in 2-4 hour exposures for 30 days but not for 60. Deposits of 100 and 50 mg./sq. ft. caused complete sterility for 14-30 and 14 days, respectively, but deposits of 10-25 mg. were mostly ineffective. Baits containing 0.5% of tepa and metepa effectively sterilized flies after aging 30-37 days on most types of surfaces. Some loss in effectiveness in tepa was apparent on metal and masonite and in metepa on wood, but none was apparent on painted wood, asphalt, metal or wax paper.

Weekly applications of cornmeal bait containing 0.5% tepa on an isolated refuse dump reduced adult house fly populations from 47 to 0 per grid count in 4 weeks and counts remained at 0 as long as the bait was distributed. The viability of eggs of female flies declined from 100 to 10% in 4 weeks and to 1% in 5 weeks. After baiting ceased, populations increased slowly but the percent viability of eggs was normal after 2 weeks. Additional small-scale field tests with cornmeal-chemosterilant baits against flies were conducted on a small garbage dump and in a poultry house. Weekly applications of apholate bait on the dump and of metepa baits in the poultry house caused some sterility and reduction of fly populations. Applications 5 days a week resulted in a high degree of control and high sterility in flies in both areas. Sterility among females was slightly higher than in males.

Comparative tests showed slight differences in the competitive ability of male flies given 1% apholate in food and those irradiated with 2850 r. Neither radiation nor the apholate completely sterilized the males but at a 4:1:1 ratio chemosterilized males caused a reduction in egg hatch of 81.4% as compared with 78% for the irradiated males.

2. Mosquitoes. In Oregon studies were conducted with a number of chemosterilants. Unfed virgin female Culex tarsalis from 1 to 6 days old did not produce eggs after being sprayed with 5% aphoxide. Adults feeding on sugar containing 0.1% apholate were completely sterilized. Females produced from larvae exposed in 1 to 3 p.p.m. of aphoxide laid numbers of viable egg masses but at 10 p.p.m. very few eggs were produced and viability was less than 1%. Adults emerging from water containing 10 p.p.m. of apholate did not lay viable eggs. Female mosquitoes were sterilized by feeding on mice which had received oral doses of 10 mg./kg. of methaphoxide or 50 mg./kg. of apholate but lower doses were not effective. Maximum effects were apparent only in adults feeding from 15-60 minutes after the mice had been treated. Studies with a radioactive chemosterilant showed that the material was rapidly absorbed by mosquitoes and mice.

Studies were also conducted to determine the effects of irradiation on various stages of <u>Culex tarsalis</u>. Dosages required to kill 100% of the various stages were as follows: Eggs, 800-1000 r; larvae, 150,000-180,000 r; pupae 80,000 r; and adults 100,000 r. Sterilizing doses were 5000 r for females and at least 10,000 r for males. Doses up to 15,000 r had no effect on adult longevity but 25,000 r was definitely harmful.

Approximately 200 chemicals and other materials were tested at the Oregon station to determine whether they would repel or attract oviposition by <u>Culex pipiens quinquefasciatus</u> or <u>C. tarsalis</u>. Several materials attracted more oviposition than distilled water but hay infusion was the most effective. A number of materials apparently were repellent and prevented oviposition by females. The most repellent materials were emulsifiers, the best of which were effective at only 2.0 p.p.m. Over 100 chemicals and other materials, including sex

extracts, were evaluated as attractants for <u>C. tarsalis</u> and <u>C. pipiens</u> quinquefasciatus. None of the materials was as attractive as the carbon dioxide standard.

3. Stable Fly. In Texas a large number of chemicals were evaluated by several methods as chemosterilants against the stable fly. most effective sterilants were apholate, aphoxide, methaphoxide and crotonamide. Topical applications of 1.8-3.7 ug. of apholate to newly-emerged flies had no effect on oviposition but viability was low (0 to 4%). Similar applications to 6-7 day old flies reduced egg viability to 2%. The sterilizing dosage was slightly less for males than for females. Flies exposed for 48 hours on residues of 10 mg./sq. ft. of apholate on glass oviposited normally but none of the eggs hatched. The residues were effective for 22 weeks. Flies exposed for 1 hour on a residue of 100 mg./sq. ft. of apholate were completely sterilized. Apholate and other sterilants were more effective on glass than on wood surfaces. Stable flies fed on blood containing 0.25 and 0.5% apholate and aphoxide and 0.125% methaphoxide oviposited normally but none of the eggs hatched. Feeding of 0.05% crotonamide did affect oviposition but only a few of the eggs hatched and none of the larvae survived to maturity. Iow concentrations of 0.001-0.002% of these 4 sterilants in larval media had no deleterious effect on larval survival or development. Larvae dipped in concentrations of 0.1 and 0.5% survived but their development was retarded.

In Texas selected chemicals and other materials were tested as stable fly attractants. None, including sex extracts, proved attractive.

Studies were initiated in Florida and Maryland on the development of physical and mechanical methods of controlling stable flies, with particular emphasis on radiant energy. Tests were conducted to develop suitable techniques for studying the response of the light and for handling the flies. The flies were equally attracted to BLB, BL and daylight fluorescent lights. The rate of attraction was not affected by changes in light intensity. Maximum response occurred near the end of the dark part of the photoperiod, indicating that a "physiological clock" may exist in flies.

4. Face Fly. In Nebraska about 200 chemicals and other materials were tested as attractants for the face fly. None of the materials were as attractive as fresh feces of cattle. Efforts to isolate and demonstrate sex attraction were unsuccessful. Preliminary studies showed that aphoxide effectively sterilized the face fly. When both sexes were fed sugar containing 0.25% aphoxide for one day or 0.0025% for three days oviposition was reduced and the eggs did not hatch. Normal females mated with males fed for 3 days on 0.005% aphoxide produced only non-viable eggs.

5. Screw-worm. The following studies were conducted at the Kerrville, Tex., laboratory. Over 250 compounds were screened as chemosterilants by several methods against various stages of the screw-worm. About 50 of the materials caused complete sterility by one or more methods of test. Nearly all of the effective sterilants were confidential materials which cannot be identified by name or structure at this time. Known materials causing complete sterility were: Apholate, aphoxide, tretamine, methaphoxide, applied topically and in adult food; colchicine, 2,6-diaminopurine and morzid in food; and metapside and Thiotepa applied topically. One material (confidential) was effective as a sterilant in the larval media but was ineffective by other means of application. Some of the active materials sterilized either sex; others were effective only when both sexes were treated; and others were effective only on one sex. Tretamine and a number of other materials sterilized all ages of flies but certain chemicals were effective only against newly-emerged flies.

There was no consistent difference in the effects of 1000 and 5000 r administered as a single dose or two equal doses. Female flies 3, 4, and 5 days old differed greatly in radiation sensitivity but dominant lethals were induced in the oocytes of all ages. In additional tests with 5-day-old pupae, which have only oogonial cells, the number of eggs deposited by females decreased as the radiation dose increased, indicating that damage to some cells were eliminated from the germ line and were not rapidly replaced.

Over 200 chemicals and other materials were screened for attractiveness to the screw-worm fly by special olfactometer procedures and by exposing the chemicals in beakers in cages of flies. In olfactometer tests isovaleraldehyde was 10 times as attractive as liver (standard), but it was less attractive than liver in beaker tests. Several additional materials were as attractive as liver but none was superior. Light increased attractiveness and maximum attraction occurred at 95° - 106° F. Materials exposed at 86° - 113° attracted 2 to 3 times as many flies as when exposed at 67° - 80° . Very few flies from 1 to 3 days responded to attractants. Highest attraction occurred when flies were 3^{-4} days old.

6. <u>Ticks</u>. Preliminary tests were conducted in Texas to study the effects of several known chemosterilants on tick molting, longevity, and reproduction. Engorged lone star tick larvae dipped in 1.0% apholate molted to nymphs and then to adults but those dipped in 0.5% aphoxide, tretamine and metepa failed to molt to nymphs. In similar tests in which engorged nymphs were dipped in 0.5% solutions the percentages molting were as follows: Apholate, 45; aphoxide, 70, and tetramine and metepa, zero. All unfed female ticks dipped in 1.0% solutions of these four materials failed to engorge. From 40-60% of those dipped in 0.5% solutions engorged but data are not yet available on the sterilizing effects of the materials. The females dipped in apholate required 15.5 days to engorge but those dipped in other materials engorged in about the same time (11.2 days) as control females.

Irradiation at the rate of 1000 r had no effect on the molting of unfed or engorged nymphs of lone star ticks. However, a dose of 2500 r prevented molting of unfed and 1-day engorged ticks and only 3% of those engorged 1 week molted to adults.

F. Evaluation of Equipment for Insect Detection and Control

- 1. Sprayers. In Texas, in cooperation with the Agricultural Engineering Research Division, one series of tests was conducted to compare the efficiency of a conventional (Bean) sprayer with that of a Spray-Foil sprayer in applying insecticides to cattle. The Spray-Foil machine gave gave slightly less control of ticks than the conventional sprayer. However, the Spray-Foil machine applied only one-tenth as much spray per cow as the conventional sprayer. With an increase of two-fold in output the Spray-Foil machine would probably give as good results as the conventional sprayer.
- 2. Mechanical Devices. In Maryland studies were initiated to evaluate available models of light traps, insect electrocutors and other mechanical devices for the control of flies and other insects. Primary emphasis has been given to comparing the attractiveness of different kinds of light and different intensities. Special test chambers were developed for this purpose. None of the devices tested proved highly attractive or effective. Further tests of existing equipment and efforts to develop more efficient traps and other devices are in progress in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions of ARS.

G. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Maryland, Mississippi, Texas, and Oregon in an effort to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. All of these studies were conducted in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the various State Experiment Stations.

In Mississippi daily applications of synergized pyrethrins sprays (0.05% pyrethrins \(\frac{1}{2} \) 0.5% synergist) with an automatic sprayer gave complete control of horn flies and significantly reduced attacks by horse flies. The effectiveness of the spray was reflected in a very low incidence (4 cases) of anaplasmosis in the treated herd as compared with that (18 cases) in the control herd. The 4 cases in the treated herd were mild, whereas there were a number of acute cases in the control herd and two animals died. The fact that first transmission in the treated herd did not occur for two months after the first case developed in the control herd serves as a further indication of the effectiveness of the sprays in protecting animals from biting flies. In another test, daily feeding of aureomycin at the rate of 0.5 mg. per

pound of body weight reduced the transmission of anaplasmosis even though no effort was made to control biting flies. Only 4 cases of the disease developed in the antibotic herd compared with 11 in the control herd. The first case in the treated herd did not occur for over two months after the first one in the control herd.

In Texas monthly surveys were continued to determine the identity and abundance of external parasites on infected (anaplasmosis) and isolated clean herds of cattle. Small numbers of lone star ticks, ear, and black-legged ticks and moderate numbers of the winter tick were present on cattle in January. Populations of the lone star tick increased steadily during February and March, but populations of other ticks were low. First horn flies appeared in March. During April, May, June and July cattle were heavily infested with lone star ticks and horn flies and with small to moderate numbers of ear tick. Populations of all these species were low during August and September. In October the winter, ear, and black-legged ticks and horn flies and grubs were present in small numbers. Moderate to high populations of the winter and ear ticks and grubs and small numbers of black-legged ticks were noted during November and December but no flies were observed on the cattle. No transmission of anaplasmosis has occurred in the isolated clean herds although no effort has been made to control potential insect and tick vectors.

The Oregon station continued surveys in Wyoming from spring to fall to determine the distribution, abundance and seasonal occurrence of potential arthropod vectors of anaplasmosis on several experimental herds of cattle on the Myers' Panch. The tick, Dermacentor andersoni, a known vector of anaplasmosis, appeared early in the spring, reached a peak population in May, declined gradually thereafter, and virtually disappeared by August. Light to moderate populations of several species of lice were present on cattle throughout the season. Horn flies and numerous species of mosquitoes were present in small to large numbers from May until the advent of cool weather. About 12 species of horse flies and deer flies were present in small numbers throughout the summer. Negative susceptible cattle have developed very few cases of anaplasmosis despite the presence of ticks and other vectors. It therefore appears that natural transmission of anaplasmosis rarely occurs under the Myers' Ranch conditions.

Studies were continued at Beltsville, Md., on the transmission of bovine anaplasmosis. Further attempts to demonstrate transovarian passage of the anaplasma agent in Dermacentor andersoni were negative. When unmated males were forced into hibernation, the survivors readily transmitted the disease 6 months and 3 weeks after engorging on infected cattle. The unmated males survived longer than mated males under hibernation and normal colony conditions. None of the mated males survived hibernating conditions. A series of D. andersoni specimens taken from cattle in the experimental areas on the Myers' Ranch in Wyoming

were tested on splenectonized calves. None of the ticks transmitted anaplasmosis.

Progress was made in studies on the anaplasmosis organism in ticks, using fluorescent antibody, electronmicroscopy, and conventional staining and histological techniques. Structures believed to be the projection part of the organism were demonstrated in the gut and feces smears by the fluorescent antibody technique. These structures were also found by electron microscopy in feces smears.

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AREA 15. SHEEP AND GOAT INSECTS

Sheep and goats are attacked by a variety of insects and Problem. ticks that are responsible for losses of many millions of dollars annually in reduced weight gains, decreased production and quality of wool, and in deaths of animals from gross attacks and insect-borne diseases. Sheep keds are a particularly serious pest in the northern States and screw-worms in the southwestern States. Fleeceworms, lice, and ticks are important pests wherever sheep and goats are raised. Safer, more effective, nonresidue-forming insecticides are needed to combat these pests. There is a special need to develop systemic insecticides that when given at low levels in feed, salt, or water would effectively control pests of sheep and goats and thereby save growers the expense of rounding up and treating flocks several times a year. New approaches to control, including attractants, chemosterilants, and radiation, should be explored and developed for controlling certain pests, as was done for the screw-worm in the Southeast. The possibilities of controlling insect pests of sheep and goats with insect pathogens, parasites, and predators also need to be investigated. Additional basic studies on the biology of the insects involved are essential for the development of biological and sanitation measures for their control. Research is urgently needed to determine which insects other than sand flies transmit bluetonque and the role of insects and ticks in the spread of other diseases of sheep and goats.

USDA PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of sheep and goats. All of the work was done at Kerrville, Texas. Studies are conducted on the biology, physiology, and food requirements of pests of sheep and goats, particularly the screw-worm and Culicoides gnats, with some attention to sheep keds and lice; on the nature of resistance to insecticides and on the length of time insecticides remain on animal skin and hair; and on the absorption, metabolism, degradation, excretion, and mechanism of action of insecticides on the insects. A recently expanded program is underway to find new ways to control pests of sheep and goats, with special emphasis on chemosterilants, antimetabolites, attractants, and noninsecticidal materials. Efforts are being made to develop adult screw-worm attractants for determining the abundance of natural populations and for use in baits for control. Research is concerned with the development of more effective contact and systemic insecticides and to study and devise sanitation or management procedures to minimize or prevent insect reproduction. Primary emphasis is given to the evaluation of new materials that leave small amounts of or no residues and to testing of formulations that will prolong effectiveness against insects and minimize toxicity hazards. Studies are conducted to determine the occurrence of residues in tissues of animals treated with insecticides in cooperation with the Animal Disease and Parasite Research Division. A limited program is being conducted on the relationship of insects to diseases of sheep and goats, involving experimental transmission from diseased to healthy animals with various species of insects, and insect surveys in epidemic areas. Current studies are centered on the insect vectors of bluetongue disease of sheep. This work is conducted in cooperation with the Animal Disease and Parasite Research Division.

The Federal scientific effort devoted to research in this area totals 4.9 professional man-years. Of this number, 1.9 is devoted to basic biology, physiology, and nutrition; 1.4 to insecticidal and sanitation control; 0.4 to insecticide residue determinations; 0.4 to insect sterility, attractants, and other new approaches to control; 0.6 to insect vectors of diseases; and 0.2 to program leadership.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State experiment stations in 1961 reported a total of 0.9 professional man-years divided among subheadings as follows: Basic biology, physiology, and nutrition 0.3; insecticidal and sanitation control 0.4; and insecticide residues 0.2.

Industry, especially chemical companies and other organizations, are engaged in research on the formulation and evaluation of insecticides for control of pests of sheep and goats. Industry also cooperates with Federal and State workers in developing information on residues resulting from the use of promising insecticides in connection with label registration. Estimated annual expenditures are equivalent to approximately 5 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

l. <u>Screw-worms</u>. Studies were made on the sexual development, behavior, and genetics of the screw-worm fly. Dissections showed that egg development was synchronous in all ovarioles. For various ages of females, the cytological condition of the nucleus of the oocyte and nurse cells was determined. The males were not vigorous in mating until 4 days old, although mating began when they were 2 days old.

In tests with various numbers of flies in holding cages, optimum longevity occurred when not over 100 adults were confined in 12" x 12" x 20" cages and when the sexes were kept separate. Excess mating by males and harassment of females reduced longevity.

Intensive efforts were made to develop a genetically distinct strain of the screw-worm fly. A black mutant male, discovered in examining many thousands of adults from scores of strains, was used to begin a black strain. A homozygous black strain was produced in this way, but it proved inferior to normal strains in longevity and mating vigor. New genes were introduced by crossbreeding with normal blue males and after several generations of inbreeding, the new black strain was equal to normal strains in mating vigor and longevity. Other genetic markers are being developed, including one in which the scutellum is fused laterally to the scutum.

Attempts to create a completely synthetic rearing medium for screwworm larvae have not yet succeeded. However, research has shown that fish flour will serve as a partial substitute for horse meat. Larvae reared for 48 hours on the standard medium (50% horse meat, 30% bovine plasma, and 20% water) completed their development after being transferred to media in which 50, 75, 87.5, and 100% of the horse meat had been replaced with fish flour. This substitution of fish flour for horse meat reduced screw-worm production costs from 21.4 to 8.7 cents per thousand pupae. The pupae produced on the substituted fish flour diet weighed less, but those produced on the 100% substitution lived slightly longer than those produced on the standard medium. In cage tests the small males mated as well as normal-sized males with normal-sized females.

Studies were made on the effects of radiation on sexual development, mating ability, and longevity of the screw-worm. Both sexes were completely sterilized by a dosage of 5,000 r and no eggs were laid; still no eggs were laid when irradiated females were mated to normal males. However, when irradiated males were mated to normal females, normal oviposition occurred, but none of the eggs hatched. A dosage of 2,000 r administered to newly emerged female flies (0-0.4 hours old) reduced ovarian growth by 50%; this dosage had no significant effect on ovarian growth of females irradiated as 5-day-old pupae. Irradiation had little or no effect on ovarian growth of females over 48 hours old. However, oocytes in 4-5-day-old females were damaged more by irradiation than those in 3-day-old females. No difference was noted in longevity of 5-, 6-, and 7-day-old flies irradiated with 7,500 r in one, two or three exposures. The number of dominant lethals and longevity of the adults was unchanged regardless of whether 5-day-old pupae were irradiated with a single dose or equal fractions at

intervals of 8 and 24 hours. Other tests showed no consistent difference in the effects of 1,000 and 5,000 r administered in two equal doses or in a single dose. Three-, 4-, and 5-day-old female flies differed greatly in sensitivity to radiation, but dominant lethals were induced in the oocytes of all three ages. In tests with 5-day-old pupae, which have only oogonial cells, the numbers of eggs deposited by females decreased as the radiation dose increased, indicating that damage to some cells eliminated them from the germ line and that they were not rapidly replaced. Decreased longevity of adults with increased dosage was noted when 4-, 5-, 6-, and 7-day-old pupae were irradiated. All stages showed lower mortality at 2,800 r than at higher dosages.

A dose of 5,000 r produces about 99% sterility when female screw-worms are irradiated as 5-day-old pupae in a well-aerated container. However, the same dosage given in an atmosphere of carbon dioxide produced less than 50% sterility. Further tests showed that irradiation in an atmosphere of 50% carbon dioxide and 50% air produced greater effects than in air alone, suggesting that the capacity of the present cobalt-60 sources could be increased by 38% by irradiating with 5,000 r in 50% air-50% CO₂. However, the insects must be held in the CO₂-air mixture for at least 30 minutes before, as well as during, irradiation.

Studies were conducted on the absorption, distribution, metabolism, and excretion of P³²metepa (chemosterilant). Metepa was absorbed faster and excreted more slowly by the stable fly than the screw-worm fly. This may account for the fact that the sterilizing dose of metepa is much less for the stable fly than for the screw-worm fly.

Mature screw-worm larvae released on soil at 73° F. in field cages entered the ground in 3-4 minutes and pupated in 24 hours. Peak emergence of adults occurred 12 days later between 5:00 and 8:00 a.m. The adult flies did not feed and exhibit mating activity until 4 days old. Most adult flies survived for 9 days but very few were alive after 22 days. At lower soil temperature (51° F.) few larvae entered the soil and although many were still alive 1 week later, none had pupated.

In studies on the dispersal and behavior of released (marked) flies, from 0.4 to 10% of the flies were recovered within 25 yards of the release point the first night. Recoveries decreased on successive nights. Dispersion was rapid in some tests, as far as 1 mile in 45 minutes; in others none apparently moved more than 400 yards. Over 90% of the adults were found resting near the tips of leafless twigs within 4 or 5 feet of the ground.

2. <u>Lice</u>. Studies of the fluctuations of normal populations of biting lice on Angora goats indicated that the lice prefer the forepart of the animal body, including the neck. Peak lice populations over a 20-week period starting November 1961 were in mid-December and the latter part of January 1962.

B. Insecticidal and Sanitation Control

- 1. Screw-worms. Research was continued to develop more effective insecticides for controlling screw-worms affecting sheep and goats. One hundred thirty one new compounds were screened for systemic action by administering them orally and subcutaneously at several dosages to quinea pigs artifically infested with screw-worms. Ten of the materials showed systemic action in one or both types of administration. One compound (Stauffer N-3054) was effective orally at 10 mg./kg. and three (Stauffer N-2310, Stauffer N-2599, and Stauffer N-3055) were effective at 25 mg./kg. All four compounds and one other (Hercules 9699) were effective at 50 mg./kg. subcutaneously; the other effective materials required higher dosages. The screw-worm larvae that survived the screening tests were reared to adults and bred to determine whether the candidate materials produced any sterilizing effects. Of 121 compounds used in this research, 13 adversely affected survival and development of resulting larvae and pupae, or oviposition and egg hatch. The most active compounds and their effects were as follows: Bayer 38636 (5 mg. orally), all male survivors; Shell 7079 (50 mg. orally), no oviposition; Stauffer R-21:04 (10 mg. orally or subcutaneously), no hatch; ENT-5734 (100 mg. subcutaneously), adults died without ovipositing; and Dilan (500 mg. orally), no adult emergence from pupae.
- 2. Sheep Nose Bots. Tests were conducted to evaluate the effectiveness of 20 of the better cattle grub systemic insecticides against nose bots in sheep. Six of the materials were highly effective.

 Materials giving 100% control of bots were as follows: Bayer 37342 as a 50 mg./kg. drench and at 50 and 100 mg./kg. in feed; Hercules 7522H as a 40 mg./kg. drench; and Dipterex as a 200 mg./kg. drench. These materials were also highly effective at lower dosages but permitted the survival of a few large third stage larvae. Materials giving 95 to 99% control were Baytex as a drench at 25 and 50 mg./kg.; Bayer 29492 orally at 25 mg./kg.; and Famophos orally at 100 mg./kg. Several other materials were highly effective against first and second stage larvae but were ineffective against large third stage larvae.
- 3. <u>Lice</u>. A number of insecticides were evaluated as dips for effectiveness against several species of goat lice, using one or two infested animals. Complete control was obtained with the following

materials: 0.15% Delnav, 0.1% V-C 13, 0.1% Dilan, 0.05% Zectran, 0.1% Geigy 30493, and 0.1% Stauffer R-1504. Other materials were less effective at the concentrations tested.

Field tests were conducted to evaluate a number of older and promising new insecticides against biting and sucking goat lice. Apparent eradication of louse infestations was obtained with sprays of 0.05% Diazinon; 0.1 and 0.25% Shell 4294; 0.25% Ruelene, V-C 13, and GC 4072; 0.5% Dilan; and Silicon dust SG-67. Sprays of 0.15% Delnav and dips of 0.25% DDT gave complete immediate control but some animals became reinfested in 1 month. Sevin at 0.1% and chlorobenzilate at 0.25% were ineffective. One flock of sheep heavily infested with biting lice was divided into two isolated groups. One group was sprayed with 0.25% V-C 13 and the other with 0.25% GC 4072. Both insecticides controlled the lice. One flock of unsheared Angora goats infested with biting lice was sprayed with 0.25% Sevin; the treatment greatly reduced but did not eradicate the lice. Seven other flocks that had been sprayed with various materials 6 months before were checked for lice at shearing time. The following failed to control the biting lice from one shearing to the next; Dilan (0.5%), Delnav (0.15%), V-C 13 (0.25%), Sevin (0.25%), Diazinon (0.05%), and GC 4072 (0.25%); however, the infestation was very light with V-C 13. Ruelene (0.25%) controlled both the biting lice and sucking lice.

- 4. <u>Biting Gnats</u>. Lindane, heptachlor, aldrin, and dieldrin were the most effective of 15 insecticides tested in the laboratory for toxicity to larvae of <u>Culicoides variipennis</u> (vector of bluetongue disease of sheep).
- 5. <u>Ticks and Keds</u>. Efforts were continued to develop more effective insecticides and other materials for controlling ticks and the sheep keds on sheep and goats. Of over 100 compounds screened for systemic effectiveness, only four showed systemic action against ticks engorging on treated guinea pigs. The effective materials, dosages (mg./kg.), and routes of administration were as follows: Rhodia RP-9895, 25 mg. orally and 50 mg. subcutaneously; Hercules 9699, 50 mg. orally and subcutaneously; Stauffer N-2310, 50 mg. subcutaneously; and Zectran, 50 mg. orally.

C. <u>Insecticide Residue Determinations</u>

1. Residue Studies. Limited studies were conducted in cooperation with the Pesticide Chemicals Research Branch to determine the amount of residues in tissues of sheep and goats after treatment with certain insecticides.

Analytical methods were developed for determining the amounts of V-C 13 in animal tissues. The method was based on the alkaline hydrolysis of the compounds and the determination of the corresponding phenols with amino antipyrene. Analyses of tissues from a sheep 2 days after being sprayed with 0.5% V-C 13 showed from 12.3 to 18.7 p.p.m. in the fat, 2.8 in the heart, 2.2 in the muscle, about 1.0 in the kidney and brain, and lesser amounts in the liver and spleen. Additional analyses were made to determine the amount of residues in the fat of sheep and goats at various intervals after spraying with 0.5% V-C 13. One week after spraying residues in the fat averaged 10.8 p.p.m. for goats. No residues were detected after 8 weeks in sheep and goats.

2. Toxicity Studies. Studies were continued in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity to livestock of insecticides and other materials applied by different routes of administration. A summary of the results is presented for the year. A detailed report will be made under Unit 2, Animal Disease and Parasites.

Extensive tests were conducted to determine the oral toxicity of a number of old and promising new insecticides to sheep and goats. tests with sheep, Co-ral at 30 mg./kg. and Shell SD 3562 at 5 mg./kg. reduced cholinesterase (ChE) by 60-80%. Dipterex caused visible toxic effects to goats at 300 mg./kg,, but not to sheep or goats at 100-200 mg./kg., with and without 25% polymer. Dipterex plus polymer reduced ChE more than Dipterex alone, but at all dosages ChE was reduced 64 to 100%. DDVP at 150 mg./kg. plus 25% polymer and Butonate at 200 mg./kg. plus 25% polymer caused no signs of toxicity but reduced ChE 77 and 47%. Dimethoate at 25 to 75 mg./kg. with and without 25% polymer produced no toxic symptoms in goats but ChE was depressed from 56 to 100%. A dosage of 150 mg./kg. was lethal. Phosphamidon at 5 mg., GC 4072 at 10 mg., Methyl Trithion and Bayer 37341 at 25 mg./kg., and Bayer 37342 at 50 mg./kg. were nontoxic to sheep but reduced ChE from 49 to 70%. Baytex at 25 mg./kg. was nontoxic, but 50 mg./kg. killed all animals. Both dosages completely depressed ChE.

Dermal applications of 0.25% Co-ral, V-C 13, and GC 4072 to sheep produced no visible toxic symptoms but reduced ChE 57 to 80%. In dip tests with V-C 13 the maximum safe and minimum toxic doses were determined as follows: sheep, 0.5 and 1.0% dip; goats, 0.25 and 0.5% dip. Little or no difference was indicated between sheep or goats shorn or in fleece. In extensive tests with Shell 4294 no symptoms of toxicity were noted in sheep or goats sprayed with 1%. In similar tests with Ruelene, the maximum nontoxic and minimum toxic concentrations were as follows: Sheep 2.5 and 5.0%; goats 1.0 and 2.5%.

When given orally the maximum nontoxic and minimum toxic dosages of Ruelene were as follows: Goats, 100 and 150 mg./kg.; sheep 150 and 200 mg./kg. Bayer 37342, given at 50 and 100 mg./kg. in feed; and at 25 and 50 mg./kg. intramuscularly caused no toxicity. Feeding the material reduced ChE somewhat more than the intramuscular injection.

Tests were also made to determine the oral toxicity of the chemosterilant, apholate, to sheep. Animals tolerated 20 weekly doses of 5 and 12.5 mg./kg. but were killed by 11 weekly doses of 20 mg./kg. A single dose of 50 mg./kg. was lethal. When given intramuscularly, apholate at 5.0 mg./kg. was lethal and at 0.5 mg./kg. death occurred after 11 weekly injections. In studies with lower dosages of apholate given orally, one sheep died after 101 daily doses of 2 mg./kg. each, but two sheep were still alive after 101 daily doses of 1 mg./kg. In general, sheep being treated showed a progress lymphocytopenia, a proportional increase in polymorphonuclear cells, and an overall leukopenia.

D. Insect Sterility, Attractants, and Other New Approaches to Control

1. Screw-worms. Over 250 compounds were screened as chemosterilants against various stages of the screw-worm. About 50 compounds gave sufficiently promising results to warrant further testing. Almost all the effective sterilants were confidential materials that cannot be identified by name at this time. Known materials causing complete sterility were: Apholate, tepa, tretamine, and metepa, applied topically and in adult food; colchicine, 2,6-diaminopurine, and morzid in food; and methiotepa and thiotepa applied topically. One material (confidential) was effective in the larval medium but ineffective by other means of application. Some of the active compounds sterilized either sex; others were effective only when both sexes were treated; still others were effective only on one sex. Tretamine and a number of other materials sterilized all ages of flies, but others were effective against newly-emerged flies.

Over 200 chemicals and other materials were screened at the Texas laboratory for attractiveness to the screw-worm by special olfactometer procedures and by exposing them in beakers in cages of flies. In olfactometer tests, isovaleraldehyde was 10-times as attractive as the liver standard but less attractive than liver in the cage tests. Several additional materials were as attractive as liver, but none was superior. Studies with liver and other materials indicated that light increased attractiveness and that maximum attraction occurred at 95° to 106° F. Materials exposed at 86°-113° attracted two to three times as many flies as when exposed at 67°-80°. Very few flies from 1 to 3 days old responded to attractants. Highest attraction occurred when the flies were 3-4 days old.

- 2. Biting Gnats. Studies were conducted on the effects of gamma irradiation on Culicoides variipennis gnats, which are vectors of bluetongue disease of sheep. Exposures to 5,000 and 10,000 r failed to produce complete sterilization in the gnats, but both dosages reduced oviposition, more noticably at the higher dosage. Males were more radiation-sensitive than females and old pupae were more sensitive than young pupae. Neither dosage caused mortality of adult Successive mating tests indicated that males gradually recovered their fertility as the percentage of infertile eggs decreased from 90 to 5-10% between the first and fifth or sixth mating. Females exposed to 15,000, 20,000 and 30,000 r were permanently sterilized but males treated as adults at 15,000 r and as pupae at 20,000 r gradually recovered fertility. Larvae were killed by 15,000 r and young pupae by 30,000 r. Males were capable of mating at least 20 times. Since they tend to recover sterility in time, even after exposure to 30,000 r, higher dosages will be required for permanent sterilization in order to utilize the sterile male release principle of control.
- 3. Ticks. Preliminary tests were conducted to study the effects of several known chemosterilants on tick molting, longevity, and reproduction. Engarged lone star tick larvae dipped in 1.0% apholate molted to nymphs and then to adults, but those dipped in 0.5% tepa, tretamine, and metepa failed to molt to nymphs. When engorged nymphs were dipped in 0.5% solutions, the percentages of molting were as follows: Apholate, 45; tepa, 70; tretamine, zero; and metepa, zero. All unfed female ticks dipped in 1.0% solutions of these four materials did not engarge. From 40 to 60% of those dipped in 0.5% solutions engarged, though engargement of those dipped in apholate required 15.5 days, as compared with 11.2 days for control females and those treated with the other chemosterilants. Viable eggs were laid by surviving females treated with any of the four chemosterilants. Considerably fewer treated ticks survived engargement than untreated ticks. One female from each of the treated groups produced egg masses that failed to hatch or egg masses with a low degree of hatch, but there appeared to be no consistent pattern of sterilization with the chemicals.

Other studies indicated that irradiation at the rate of 1,000 r had no effect on the molting of unfed or engarged nymphal lone star ticks. However, a dose of 2,500 r prevented molting of unfed and 1-day engarged ticks and only 3% of those engarged 1 week molted to adults.

E. Insect Vectors of Diseases

l. Biting Flies and Gnats. Studies were continued, in cooperation with the Denver, Colo. laboratory of the Animal Disease and Parasite Research Division, on the transmission of bluetongue disease of sheep. Eighteen species of biting flies were collected in a trap baited with a living sheep, including five species of Culicoides, four species of Simulium (black flies) and six species of mosquitoes. Eight specimens of Culicoides variipennis were taken in the process of engorging on the sheep. Before shearing, adults of variipennis were seen flying and crawling about the sheep. After shearing, one of them fed by crawling into the short hair covering the belly-the remaining seven fed at a tiny bare area where the sheep's belly was nicked during the shearing. At five ranches where bluetongue occurred, C. variipennis was taken in small numbers.

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AREA 16. POULTRY INSECTS

Numerous species of insects, mites, and ticks are common pests Problem. of poultry throughout the country and if not controlled can make poultry raising unprofitable. They cause poultry to look unsightly, reduce weight gains and egg production, and mar the skin, which results in downgrading of quality and lower market prices. Pests such as black flies and mosquitoes transmit leucocytozoon and fowl pox diseases which exact a heavy toll in death and unthrifty poultry each year. House flies spread parasites and enteric diseases which may decimate flocks. more effective, nonresidue-forming insecticides are needed to combat these poultry pests and vectors of diseases of poultry. Better materials are needed for direct application to poultry or in poultry houses to control lice, mites, and ticks and for use as larvicides or fly baits to control flies. Materials are especially needed which, when given in feed or water, would act systemically to control external pests and render droppings toxic to fly larvae. Exploratory studies are needed to investigate possibilities of developing attractants, chemosterilants, antimetabolites, or other new methods of combatting poultry pests. Biological and sanitation methods of control offer excellent possibilities for control and need to be emphasized. There is a special need to investigate the roles of insects, ticks, and mites in the transmission of poultry diseases.

USDA PROGRAM

A continuing study is underway involving basic and applied research on insects, mites, and ticks that affect the health and productivity of poultry. Studies are designed to determine breeding habits and reproductive capacities of various poultry pests and to gain further knowledge on the nature of resistance of these pests to certain insecticides. Work at present is devoted mostly to lice and the northern fowl mite, and to the house fly, which breeds abundantly in poultry droppings. A newly expanded program aims to find new ways to control pests of poultry with special emphasis on chemosterilants, antimetabolites, attractants, and noninsecticidal materials and methods. Current studies in this field are largely limited to house flies.

Some attention is being given to the development and testing of <u>attract-ants</u>; and <u>physical and mechanical methods</u> of controlling house flies are being investigated in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions.

Research is concerned with the development of more effective <u>insecticides</u> for the control of poultry pests. New chemicals are screened for contact and residual toxicity to lice and mites attacking poultry and to house

flies, and promising ones are tested for effectiveness under practical field conditions. New methods of utilizing insecticides more efficiently and safely are being investigated, with special attention to finding materials that, when given orally in water or feed, will act systemically to kill lice and mites on the poultry, and render the droppings toxic to fly larvae. Efforts are also being given to methods of sanitation and management to control fly breeding in accumulations of manure in poultry houses. Studies are conducted to determine the occurrences of residues in tissues of poultry treated with insecticides. Work is done in cooperation with poultry raisers at Orlando, Fla., Stoneville, Miss., Corvallis, Oreg., and Kerrville, Tex.

The <u>Federal</u> scientific effort devoted to research in this area totals 2.2 professional man-years. Of this number, 0.4 is devoted to <u>basic</u> biology, physiology, and nutrition; 0.9 to <u>insecticidal</u> and <u>sanitation</u> control; 0.2 to <u>insecticide</u> residue determinations; 0.6 to <u>insect</u> sterility, attractants and other new approaches to control; and 0.1 to program leadership.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 3.3 professional man-years divided among subheadings as follows: Sasic biology, physiology, and nutrition 0.5; insecticidal and sanitation control 1.8; insecticide residues 0.8; insect sterility, attractants, and other new approaches to control 0.1; and insect vectors of disease 0.1.

Industry, especially chemical companies and distributor companies, are engaged in research on formulation and evaluation of insecticides for control of poultry pests. Industry also cooperates with Federal and State workers in developing information on residues resulting from the use of promising insecticides in connection with label registration. The cooperation of poultry raisers in providing large numbers of poultry is of inestimable value in the field evaluation of insect control by Federal and State workers and represents a significant contribution by poultrymen to the total research efforts. Estimated annual expenditures by industry are equivalent to approximately 5 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology and Physiology

1. House Fly. Studies in Oregon showed that DDT-resistance in house flies was attributable to the ability of the flies to dehydrochlorinate the insecticide. The mechanism of resistance in house flies to the carbamates was due to aliesterase activity - the same as for the organophosphates.

In Oregon certain synergists (simple tris-substituted derivatives of phosphoric acid) completely overcame high levels of malathion resistance in house flies. The most effective materials increased the toxicity of malathion from 36- to 40-fold against the resistant flies. The relative ability of several of the synergists to synergize malathion against the resistant flies (little or no effect with normal flies) was directly related to the inhibitory effect of the synergists on ali-esterase activity. The synergists may actually inhibit the mutant ali-esterase present in all organophosphate-resistant fly strains. House flies treated with the synergist tributyl phosphorotrithioate and then treated with either parathion or paraoxon accumulated greater quantities of paraoxon than did flies treated with the toxicants only.

In Oregon selection of a house fly colony with Isolan produced a strain with 3-fold resistance in 14 generations. At the same time, levels of esterase activity to methyl butyrate declined to 40% of the original level in flies of the selected strain. This same phenomenon occurs when house flies are selected with organophosphates, indicating that the same mechanism is responsible for resistance to both classes of insecticides.

Studies in Oregon showed that house flies are capable of dispersing a distance of at least 5 miles in 24-48 hours.

In Florida cytological studies showed that the ovaries in normal 3-day-old flies were 6-8 times as large as those in females fed for 3 days on 1.0% apholate. After feeding ceased some growth occurred but ovaries never attained normal size. Females mated to males fed on 0.4-1.0% apholate oviposited but the eggs showed little or no embryonic development, whereas females mated with males fed 0.1-0.3% laid some viable eggs and some without embryonic development. Similar results were obtained in tests with normal and insecticide-resistant strains of flies.

In further studies on the cytological effects 1% apholate given in adult food over a period of 24 hours inhibited but did not eliminate ovarian development in females. The greatest effect was noted at 72 hours after eclosion of the nurse cells in the first and second egg chambers. The chromatin was irregular and nuclei had bizarre shapes. Occytes matured in the first cell but not in the others. The germarium was also affected as the third egg chamber was not visible until 168-192 hours after eclosion compared to 96 hours in normal flies.

2. Mosquitoes. Studies were initiated at Fresno, Calif., in July, 1961 on the biology of mosquitoes in relation to agriculture, especially with regard to irrigation and land management practices, in cooperation with the Soil and Water Conservation Research Division and The Bureau

of Vector Control of the California State Department of Health. Early studies showed that dairy drains are sites of heavy breeding of <u>Culex quinquefasciatus</u> through November and into early December. The breeding in such locations contributes heavily to overwintering adult population of this species. No autogeny (ability to lay eggs without a prior blood meal) was observed in this vicinity with <u>Aedes vexans</u>, <u>Culex apicalis</u>, <u>C. peus</u>, and <u>C. thriambus</u>.

In Oregon, studies on flight movements of <u>tarsalis</u> indicated that they move from their resting stations about sunset and return about sunrise. The instinct of <u>tarsalis</u> to oviposit in low sites is stronger than the instinct for oviposition in favorable waters. Female <u>tarsalis</u> mate only once, whereas males mate several times.

In other studies in Oregon on the physiology of resistance, malathionresistant larvae of Culex tarsalis were more efficient in regulating salt (chloride) uptake than susceptible larvae during exposure to malathion. Resistant and susceptible larvae take up similar amounts of chloride when exposed to 1% sodium chloride alone. Exposure to 1% sodium chloride resulted in an increase in oxygen consumption in susceptible and malathion-resistant larvae, but chloride had no measurable effect on cholinesterase inhibition or accumulation of malaoxon, the principal breakdown product of malathion in mosquitoes. Studies of the insect enzyme systems, cholinesterases and aliesterases, led to the development of synergists for organophosphorus insecticides. eral tris-substituted derivatives of phosphoric acid overcame the resistance of tarsalis larvae to malathion, increasing its toxicity 100-fold to a resistant strain. Only about a 2-fold increase was indicated against normal strains. The ability of the synergists to overcome resistance appears to be related to an increase in the titer of a carboxyesterase enzyme. In general, the accumulation of malaoxon was proportionate to the effectiveness of the synergist. Malathionresistant tarsalis detoxify DDT 2- to 3-times as fast as normal strains, largely through the formation of carboxylic derivatives. The DDT detoxifying enzyme, DDT-dehydrochlorinase, in flies does not occur in tarsalis.

Studies in Florida showed that metepa was picked up rapidly by tarsal contact from glass surfaces by both mosquitoes and house flies. House flies and Aedes quadrimaculatus absorbed approximately the same amount (7 µg./insect) during a 4-hour exposure on surfaces treated at 10 mg./-sq. foot. The quadrimaculatus males were so damaged that they could not inseminate the females but house fly males were normal in this respect and 99% of the eggs produced from matings with virgin untreated females were nonviable. A. aegypti showed an average uptake of 2.5 µg./insect, which sterilized but apparently did not impair male activity. Feeding for 3 days on treated food (1% in 20% honey solution) with

mosquitoes, and 0.4% in house fly food sterilized quadrimaculatus males and caused a high degree of sterility in house fly and aegypti males. At the end of the 3-day feeding period the amount of tagged material, expressed at ug.-equivalents of P32 metepa was 3.0, 3.7, and 1.7 in males of quadrimaculatus, house flies, and aegypti, respectively. Exposure of larvae from the third instar through pupation in water treated at 10 p.p.m. failed to sterilize quadrimaculatus or aegypti significantly and the uptake of metepa was low in comparison to the other types of treatments.

Studies in Oregon showed that P32 metepa was rapidly absorbed and metabolized by house flies, mosquitoes (<u>Culex tarsalis</u>) and mice. Almost complete degradation occurred in 24 hours with phosphoric acid being the major metabolic product.

In the field in central Florida the number of Anopheles quadrimaculatus adults generally decreased in January and February and increased little in March, April, and May (water level low). The counts increased in the last quarter of the year, even though rainfall was deficient and water levels remained low. The females in the breeding area generally outnumbered the males, often better than 2:1. Throughout the year some of the eggs laid by wild females failed to hatch, indicating some sterility in nature. The sterility in nature appeared highest in September and October. Two wild females that had laid eggs had no sperm. A higher percentage of the females collected at the breeding site had laid two or more batches of eggs than those found away from the water. Studies with laboratory colony males and females indicated males were capable of fertilizing about 1.5 females each in a 3-day period.

B. <u>Insecticidal and Sanitation Control</u>

1. House Fly. In Florida, approximately 175 new compounds were screened for residual effectiveness against house flies. Twenty-four materials were 98-100% effective for at least 24 weeks as compared with 12-16 weeks for malathion (standard). These were Thiodan, General Chemical GC-3583, Shell SD-4402, Upjohn TUC U-12927 (with synergist); Bayer compounds 29952, 30237, 30468 and 29492; Hooker HRS-1422, Geigy G-27365, Stauffer N-2404, heptachlor epoxide; and 12 confidential compounds. Bayer 29952, Bayer 30237 and seven of the confidential compounds were still 100% effective after 44-48 weeks and are considered unusually promising for controlling flies. A number of promising new compounds were evaluated as space sprays against susceptible and resistant strains of house flies. Several of the compounds were superior to malathion (standard) against susceptible flies but only one, Bayer 30911, was superior against resistant strains.

In Florida, over 50 selected compounds were evaluated as bait toxicants against resistant and normal strains of house flies. Eight of the compounds were equal or superior to Dipterex (standard) against both strains of flies, namely: Bayer 30237, Bayer 30554, General Chemical 6506, Stauffer N-2230, Stauffer N-2404, dimethoate, and two confidential compounds. All of the compounds tested were more effective against normal than against resistant flies.

In Florida several materials which increased the effectiveness of malathion in laboratory tests were evaluated as residual treatments in barns against material populations of flies. Combinations of several synergists and malathion alone were equally ineffective in these tests.

2. Mosquitoes. Research was continued by the Orlando, Fla., and Corvallis, Oreg., stations to develop more effective insecticides and other materials for controlling mosquitoes. In Florida, of over 500 new compounds screened for toxicity to Anopheles quadrimaculatus larvae, five were outstanding in their effectiveness. Of seven selected compounds screened against Culex tarsalis in Oregon, three were outstanding.

Studies were continued in Florida to find more effective residual insecticides for the control of adult mosquitoes. Of 157 selected compounds screened against female adults of Anopheles quadrimaculatus, 17 were very promising, causing 90 to 100% kill of exposed quadrimaculatus for 24 weeks. Four of the compounds, Bayer 39007, Hercules 7522C, Bayer 34098, and Bayer 4183 retained their effectiveness on sprayed surfaces for 1 year. In tests with treatments that had aged for 2 years, Sevin and Hercules AC-5727 plus piperonyl butoxide caused 85% kills of quadrimaculatus within 24 hours after a 1-hour exposure to 100 mg./square foot, whereas malathion and unsynergized AC-5727 were ineffective.

In Florida, tests in the laboratory and field were continued to develop more effective materials for mosquito control. Of 151 selected compounds tested as space sprays against colonized Aedes taeniorhynchus, four were better than the malathion standard: Kenya Oleo Resin, Bayer 34042, Bayer 30749 and Hercules 7522C. In aerial spray tests against natural populations of salt-marsh mosquito adults, 0.05 pound per acre of Dibrom or DDVP was about as effective as 0.1 pound of malathion per acre. These sprays reduced mosquito abundance more than 90% in 6 hours.

In Oregon, screening tests were conducted with a number of new insecticides against larvae and adults of <u>Culex tarsalis</u>. Three of the materials gave 100% kills of larvae at a concentration of only 0.025 p.p.m. In spray tests seven materials were 2 to 9 times more toxic to adult mosquitoes than malathion (standard) and two of these compared favorably with parathion.

3. Lice and Mites. In comparative tests with various insecticides as sprays in Mississippi, Zectran at 0.025, Hooker HRS-1422 at 0.05, and Shell SD 4294 (Ciodrin) at 0.1% gave complete control of the northern fowl mite and lice. When tested by dipping only the tails of chickens, Sevin at 0.5% was the most effective material against mites and lice. However, malathion at 0.5 and GC-4072 at 0.25% eradicated lice and greatly reduced mite infestations. In field flock tests, tail dipping with 0.25 and 0.5% Sevin eradicated mite infestations. When applied as litter treatments, I pound of 5% Zectran or Sevin dusts per 80 square feet failed to give consistently satisfactory control of mites but were effective against lice. However, 50% dusts of Sevin applied in pits and nest boxes eliminated mite infestations. Four weekly sprayings of coops and roosts eliminated the northern fowl mite in one location, but not in seven others, although high reductions in populations occurred. Feeding Ruelene to chickens at rates of 10-30 mg./kg. for 3 days had no effect on louse or mite populations.

Eleven chemicals were tested in Texas for control of poultry lice on chickens. Nine of the compounds and the malathion standard controlled all species of lice through a 27-day period at levels as low as 0.1%. Dibrom was effective at 0.25%. The materials were applied as sprays directly to the individual birds.

C. Insect Sterility, Attractants, and Other New Approaches to Control

1. House Fly. Studies in Oregon indicated that irradiation of resistant flies with sub-sterilizing doses of 1000 r did not alter their susceptibility to insecticides or esterase activity. Treated females mated with untreated males oviposited normally but only 45% of the eggs hatched. Additional studies were conducted with normal and parathion-resistant flies that had been irradiated as pupae with 600 r for seven generations. Only about 25% of the eggs hatched from matings of irradiated males and females of either colony. No changes in insecticide susceptibility or esterase activity were apparent.

The following studies were conducted in Florida in connection with control of the house fly in dairy barns, poultry houses, households and industrial establishments. Irradiation caused greater damage to early (2-4 days old) house fly pupae than to middle-age pupae. Virtually no damage was apparent in old pupae and longevity of adults was greater than that of younger pupae. Irradiation of pupae 0-36 hours before adult emergence did not produce complete sterility and some recovery was indicated since second matings resulted in a slight increase in progeny. No recovery occurred in pupae irradiated 72-96 hours before adult emergence.

Extensive studies were conducted to find materials that would induce sterility or otherwise affect the growth and development of the house fly. Of about 1300 compounds tested in the adult food, twenty-one

caused sterility in flies. Only one of about 800 materials tested in the larval media caused sterility but nearly one-third were toxic to the larvae. In secondary tests with 59 compounds that had shown promise in screening tests, twenty-seven caused sterility (no oviposition or hatch) at concentrations of 1% or lower. Additional tests were run with 50 promising materials applied as larval dips, topically to adults, and in the adult food. None caused sterility as larval dips but in adult food, six induced complete or nearly complete sterility. Two other materials were effective only as topical applications.

Special tests were run with 24 promising chemosterilants to ascertain the effects on each sex. Only one material, an aziridinyl compound, caused sterility in both sexes. Methiotepa, 5-fluoroacetic acid, metepa, and a confidential material caused complete sterility in males but not always in females. Three materials - 5-fluoroacetic acid, an aziridinyl compound, and 5-fluorouracil were effective when fed to both sexes.

Extensive studies were conducted to learn more about the action of several effective chemosterilants on house fly sexual development, mating and reproduction. In one series of tests, males sterilized by feeding 3 days on apholate proved fully competitive with normal males when placed with normal females. When only treated males were placed with normal females all eggs were sterile and 12.5% were sterile when only normal males were present. When normal females, normal males, and treated males were combined at ratios of 1:1:1 and 1:1:2. 65 and 80% of the eggs were sterile and higher ratios of 1:1:3, 1:1:5, and 1:1:10 resulted in 99.9-100% sterility. Additional tests at these ratios confirmed that actual sterility was higher than the expected. Males given food containing 0.4-1.0% apholate for 3 days were sterilized for life but lower concentrations of 0.1-0.3% were not 100% effective. Exposures of males on residues of 500-1000 mg./kg. of tepa on plywood panels caused only partial sterility (12-72%) but when applied at 250-500 mg./sq. ft. with sugar, tepa and apholate produced 91-100 and 99-100% sterility, respectively. When applied in glass jars, residues of 250 mg./sq. ft. of tepa or metepa completely sterilized flies in 2-4-hour exposures for 30 days but not for 60. Deposits of 100 and 50 mg./sq. ft. caused complete sterility for 14-30 and 14 days, respectively, but deposits of 10-25 mg. were mostly ineffective. Baits containing 0.5% of tepa and metepa effectively sterilized flies after aging 30-37 days on most types of surfaces. Some loss in effectiveness in tepa was apparent on metal and masonite and in metepa on wood, but none was apparent on painted wood, asphalt, metal or wax paper.

In Florida weekly applications of corn meal bait containing 0.5% tepa on an isolated refuse dump reduced adult house fly populations from 47 to 0 in 4 weeks and counts remained at 0 as long as the bait was distributed. The viability of eggs of female flies declined

from 100 to 10% in 4 weeks and to 1% in 5 weeks. After baiting ceased, populations increased slowly but the percent viability of eggs was normal after 2 weeks. Additional small-scale field tests with corn meal-chemosterilant baits against flies were conducted on a small garbage dump and in a poultry house. Weekly applications of apholate baits on the dump and of metepa baits in the poultry houses caused some sterility and reduction of fly populations. Applications 5 days a week resulted in a high degree of control and high sterility in flies in both areas.

Mosquitoes. In Florida, extensive studies were conducted with the chemosterilants, apholate, tepa, and metepa. In laboratory tests with Anopheles quadrimaculatus and Aedes aegypti sterility could be induced into both males and females by feeding the chemicals in adult food (honey - water), by allowing adults to walk on deposits on glass or masonite surfaces (tarsal contact) or by exposing larvae to the materials in water solutions. However, two of the three compounds were not effective in one of the three methods of administration: apholate did not sterilize by tarsal contact and metepa did not sterilize when larvae were exposed to it. A residual deposit of tepa sterilized males from the laboratory colony that were 0 to 5 days old, females from the laboratory colony that were 0 to 24 hours old, and wild-collected females that had previously mated, had a blood meal, and developed one to five batches of eggs. In general, depending on the dosage, treated females either had stunted ovaries and laid no eggs or laid eggs which did not hatch. When treated males were mated to untreated females, the females received motile sperm and laid normal numbers of eggs which did not hatch. In some cases with either treated males or females, an occasional egg hatched. Most of the eggs laid by females mated to treated males showed no or disorganized development; some eggs embryonated normally, but did not hatch. Artificial resting stations for A. quadrimaculatus were established in a breeding area in the field and treated with tepa. These deposits were effective in sterilizing wild females that rested in them (males could not be studied, but presumably were sterilized) completely; however, the deposits remained effective for only a few days. sterility was found in females collected outside the treated boxes, indicating that this method did not affect a sifnificant part of the total population. Release of gamma- or chemosterilized A. guadrimaculatus males into natural populations have been ineffective to date in influencing wild populations.

In Oregon studies were conducted with a number of chemosterilants. Unfed virgin female <u>Culex tarsalis</u> from 1 to 6 days old did not produce eggs after being sprayed with 5% tepa. Adults feeding on sugar containing 0.1% apholate were completely sterilized. Females produced from larvae exposed in 1 to 3 p.p.m. of tepa laid normal numbers of viable egg masses but at 10 p.p.m. very few eggs were produced and

viability was less than 1%. Adults emerging from water containing 10 p.p.m. of apholate did not lay viable eggs. Female mosquitoes were sterilized by feeding on mice which had received oral doses of 10 mg./kg. of metepa or 50 mg./kg. of apholate but lower doses were not effective. Maximum effects were apparent only in adults feeding from 15-60 minutes after the mice had been treated. Studies with a radioactive chemosterilant showed that the material was rapidly absorbed and metabolized by mosquitoes and mice. Almost complete degradation occurred in 24 hours.

In Oregon studies were conducted to determine the effects of irradiation on various stages of <u>C</u>. <u>tarsalis</u>. Dosages required to kill 100% of the various stages were as follows: eggs, 800-1000 r; larvae, 150,000-180,000 r; pupae 80,000 r; and adults 100,000 r. Sterilizing doses were 5000 r for females and at least 10,000 r for males. Doses up to 15,000 r had no effect on adult longevity but 25,000 r was definitely harmful.

In Oregon approximately 200 chemicals and other materials were tested to determine whether they would repel or attract oviposition by <u>Culex pipiens quinquefasciatus</u> or <u>C. tarsalis</u>. Several materials attracted more oviposition than distilled water but hay infusion was the most effective. A number of materials apparently were repellent and prevented oviposition by females. The most repellent materials were emulsifiers, the best of which were effective at only 2.0 p.p.m. Over 100 chemicals and other materials, including sex extracts, were evaluated as attractants for <u>Culex tarsalis</u> and <u>C. pipiens quinquefasciatus</u>. None of the materials was as attractive as the carbon dioxide standard.

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AREA 17. INSECTS AFFECTING MAN, HOUSEHOLDS, AND INDUSTRIAL ESTABLISHMENTS

Insects, ticks, and mites are responsible for widespread human misery and certain insects cause heavy losses of food and materials in households and industrial establishments. Many of the same or closely related insects which affect man are also important pests of livestock, thus research on insects in relation to man and to livestock is mutaully advantageous. Certain arthropods are vecotrs of major diseases which annually cause the deaths of millions of humans. Mosquitoes, for example, transmit malaria, dengue, encephalitis, yellow fever, and filariasis. Some of these diseases, as well as other arthropod-borne diseases, occur and are potentially serious problems in the United States but most of them are of more concern in other parts of the world, where troops and civilian personnel are maintained. The military agencies have for many years depended on the research competence in agriculture for answers to their military insect and insect-borne disease problems. Attacks by insects, ticks, and mites frequently prevent farm and forest work, reduce or destroy the value of recreation areas, and even make certain areas uninhabitable. Property values are often depressed and development prevented by hordes of annoying pests. Mosquitoes, bed bugs, and fleas are frequently serious annoyances in homes. Other household insects are of economic importance in homes and industrial establishments because they damage foods, fabrics, and other materials, causing losses of millions of dollars annually. There is a great need for safe, economical insecticides and satisfactory methods for their application that could be used quickly and effectively to control local infestations or outbreaks of pests that annoy man in the field or at home, especially where there are threats of disease epidemics. Improved means for controlling mosquitoes, sand flies, gnats, the imported fire ant, and similar pests, should receive particular attention. More efficient repellents are needed to protect humans, particularly when other means of control cannot be employed. Special efforts should be made to develop systemic materials which when taken orally would repel or prevent insects from biting. Sanitation, habitat management, and other noninsecticidal methods of control should be reappraised, and biological control, especially with insect pathogens, needs to be fully explored. New approaches to control, including chemosterilants, antimetabolites, attractants, and radiation require intensive investigation. Studies should be undertaken on the biology. ecology, physiology, and genetics of many important pests affecting man and the household in order to find weak points in their life cycles which might be utilized to improve control efficiency.

USDA PROGRAM

The Department has a continuing program involving applied and basic research on insects affecting man, households and industrial establishments, including mosquitoes, house flies, human lice, bed bugs, anats, fleas, ticks, mites and other pests of man, and on cockroaches, ants, and several other pests of importance in households and industrial establishments. Research is directed toward the development of more effective insecticides and repellents and involves primary screening of chemicals and field evaluation of promising materials. Investigations are conducted on the nature of insect resistance to insecticides, on the mode of action of insecticides, on the effects of radiation and chemosterilants, on attractants, on factors affecting attraction of biting insects to humans, and on the factors affecting the effectiveness of repellents. Attention is also given to the development of sanitation and management procedures and to biological control methods for mosquitoes, house flies, cockroaches and several other pests. Studies are conducted in cooperation with the Agricultural Engineering Research and Animal Husbandry Research Divisions to develop physical and mechanical methods of insect control and to evaluate various kinds of traps and devices for estimating and controlling natural populations of flies, mosquitoes, and other pests. Studies are also conducted in cooperation with the Soil and Water Conservation Research Division of ARS and the Bureau of Vector Control, California State Department of Health, on soil and water management procedures to prevent mosquito production in Western irrigated areas and in log ponds. The major portion of the program is conducted at Orlando, Fla; the remainder of the work is done at Corvallis, Oreg., Reno, Nev., Fresno, Calif., and Beltsville, Md. The station at Reno, Nev. was closed in September 1961 and the work transferred to Fresno, Calif., to permit expansion of research on irrigation mosquitoes.

The <u>Federal</u> scientific effort devoted to research in this area totals 20.1 professional man-years. Of this number 3.1 is devoted to <u>basic</u> biology, physiology, and nutrition; 9.1 to <u>insecticidal</u> and <u>sanitation control</u>; 0.7 to <u>biological control</u>; 5.7 to <u>insect sterility</u>, attractants and other new approaches to control; 0.5 to the <u>evaluation</u> of equipment for insect detection and control; and 1.0 to <u>program leadership</u>.

Additional research (2 professional man-years) has been initiated under a grant of P.L. 480 funds to the Facultad de Agronomia, Universidad de la Republica, Uruguay, on "Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay."

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 14.7 professional man-years divided among subheadings as follows: Rasic biology, physiology, and nutrition, 7.0; insecticidal and sanitation control, 4.3; biological control, 1.2; insect sterility, attractants and other new approaches to control, 1.1; and evaluation of equipment for insect detection and control, 0.6. Industry and other organizations, including county organizations and farm owners, cooperate in research studies by supplying test sites, equipment, and chemicals. Industry has contributed substantially through the synthesis, analysis, formulation, and primary screening of candidate insecticides. There are consultation and mutual cooperation between Government scientists and industry in developing new materials for label registration and practical use. Estimated annual expenditures by industry are equivalent to approximately 10 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Orlando, Fla., failure to induce sterility in or to reduce wild populations of Anopheles quadrimaculatus through the release of laboratory-reared males made sterile by radiation or chemicals prompted studies on the population levels, degree of insemination of females, viability of eggs, physiological age, and mating behavior of the mosquitoes under field conditions. Studies were initiated in an area where adult populations were low and females were over twice as numerous as males. A high percentage of females collected in the breeding area and in resting stations outside the breeding area, were inseminated throughout the year. Some of the eggs laid by wild females failed to hatch, indicating that some sterility occurs in nature.

The physiological age of female <u>quadrimaculatus</u> collected in the central Florida test area was determined by dissecting the ovaries and counting the follicular relics in individual ovarioles. The oldest female found during the year-long study had laid 6 batches of eggs. Half or more of the females collected each week had laid only one batch of eggs, indicating that the population was continually replacing itself and was a comparatively young population. Tagged virgin females from the laboratory colony were released alone or with laboratory males sterilized by radiation or chemicals to study the mating behavior of wild <u>quadrimaculatus</u> populations. The recoveries of released females ranged from 0.½ to 22%. From 36 to 80% of the females from the laboratory colony were mated by wild males within 3 to 4 hours after release. Eggs from laboratory females mated with

Wild males were viable and progeny were reared to the adult stage. These progeny mated poorly in laboratory cages. When tagged virgin laboratory females and laboratory males sterilized with apholate, aphoxide, or gamma radiation were released into the field, viability data on eggs from recovered laboratory females showed competition between fertile wild males and sterile laboratory males. Fertile wild male mating was greater near known breeding areas than at short distances away. When metepa-sterilized laboratory males were released daily over a two-week period close to breeding areas, no sterility was found in wild females collected from the area of release, indicating that the laboratory males did not mate readily with wild females. The objectives of this work are to develop techniques to measure and study biological and mating behavior of mosquitoes under field conditions and to determine the feasibility of release of sterile males for control or eradication.

Very few wild <u>quadrimaculatus</u> females could be collected by the usual methods of light traps or inspection of buildings. Of many collecting methods tried, the most productive one was aspirating females from a parked vehicle while the motor was still warm.

Under laboratory conditions, laboratory and wild strains of Anopheles quadrimaculatus differed markedly in their response to light and mating activity. Wild adults left hiding places at lower light intensisties than laboratory individuals. Laboratory adults mated but wild adults did not.

Studies at Reno, Nev., confirmed earlier observations that abandoned mines furnish excellent overwintering habitats for mosquitoes, especially Culex tarsalis, a vector of encephalitis. Ten species mosquitoes from such locations were autogenic (capable of oviposition without a blood meal): Aedes campestris, A. communis, A. dorsalis, A. melanimon, A. niphadopsis, A. nigromaculis, A. schizopinax, Culex erythrothorax, Culex tarsalis, and Culiseta incidens. Only Aedes communis and Culex tarsalis had previously been reported autogenic. Population emergence for Culex erythrothorax, which overwinters as larvae, was 1-1/2 to 2 months earlier in southern Nevada than in northern and central areas of the State. In two breeding areas heavy ice and destruction of emergent vegetation by migratory ducks and geese reduced the larval population noted the previous November by about 97%. In other areas a gradual build-up of pupae was observed in April, and pupae predominated in late April and early May. colony of this species had been established in the laboratory. campestris, believed to be a univoltine species (one brood per year) produced a large brood in June following heavy rainfall. this species breeds only in the spring. Biting adults of Anopheles freeborni were noted in early February in southern Nevada; some

Culex tarsalis activity was noted in late March in the valleys. Only a few Aedes niphadopsis could be induced to feed on humans, although this species has been reported a vicious biter of livestock and humans. Breeding of Aedes increpitus began at an elevation of μ ,500 feet early in February and at 6,300-6,700 feet in late March. In the spring a survey of 74 mosquito breeding sites showed Aedes niphadopsis the dominant species in 65%, Aedes campestris in 28%, and Aedes dorsalis in 7% of the sites.

Surveys were continued on biting arthropods of the Humboldt River Basin (Nevada) in connection with a major study of water resources being carried out cooperatively by the U. S. Departments of Agriculture and Interior, the University of Nevada, and the Nevada State Department of Conservation and Natural Resources. For the third successive year water flow was only about one-fourth of normal and very few breeding areas received water. Only light breeding of Aedes melanimon, A. vexans, Culex tarsalis, and Culiseta inornata was observed. Very few biting adult mosquitoes were noted. Tabanids (horse and deer flies) and black flies were also negligible.

At Fresno, Calif., cooperation with the Soil and Water Conservation Research Division and the California Bureau of Vector Control will permit expansion of the research on control of mosquitoes in relation to irrigation practices. Early studies showed that dairy drains are sites of heavy breeding of <u>Culex quinquefasciatus</u> through November and into early December. The breeding in such locations contributes heavily to overwintering adult populations of this species. No autogeny (ability to lay eggs without a prior blood meal) was observed in this vicinity with <u>Aedes vexans</u>, <u>Culex apicalis</u>, <u>C. peus</u>, and <u>C. thriambus</u>.

At Corvallis, Oreg., laboratory studies indicated that fourth instar Aedes sierrensis larvae enter diapause from about the last week in September through the third week in December; diapause was induced through manipulations of the photoperiod and broken by constantly increasing day lengths. Studies on flight movements of tarsalis indicated that these mosquitoes move from their resting stations about sunset and return about sunrise. The instinct of tarsalis to oviposit in low sites was stronger than the instinct for oviposition in favorable waters. Female tarsalis mated only once, whereas males mated several times.

In Oregon, studies were continued on the physiology of insect resistance to insecticides. Malathion-resistant larvae of <u>Culex tarsalis</u> were more efficient in regulating salt (chloride) uptake than susceptible larvae during exposure to malathion. Resistant and susceptible larvae took up similar amounts of chloride when exposed to 1% sodium chloride alone. Exposure to 1% sodium chloride resulted

in an increase in oxygen consumption in susceptible and malathionresistant larvae, but chloride had no measurable effect on cholinesterase inhibition or accumulation of malaoxon, the principal breakdown product of malathion in mosquitoes. Studies of the insect enzyme systems, cholinesterases and aliesterases, led to the development of synergists for organophosphorus insecticides. Several trissubstituted derivatives of phosphoric acid overcame the resistance of tarsalis larvae to malathion, increasing its toxicity 100-fold to a resistant strain. Only about a 2-fold increase was indicated against susceptible strains of tarsalis. The ability of synergists to overcome resistance to malathion in tarsalis appears to be caused by an increase in the titer of a carboxyesterase enzyme. In general, the accumulation of malaoxon was proportionate to the effectiveness of the synergist. Malathion-resistant C. tarsalis mosquitoes detoxify DDT 2-3 times as fast as normal strains, largely through the formulation of carboxylic acid derivatives. The detoxifying enzyme.DBTdehydrochlorinase does not occur in tarsalis.

Studies were continued in Oregon to determine the effects of radiation on various stages of <u>Culex tarsalis</u>. The dosages required to kill 100% were as follows: Eggs, 800-1000 r; larvae, 150,000-180,000 r; pupae, 80,000 r; and adults, 100,000 r.

Studies in Florida showed that a chemosterilant, metepa, was picked up rapidly by tarsal contact from glass surfaces by mosquitoes. Anopheles quadrimaculatus absorbed approximately 7 µq./insect during a 4-hour exposure at 10 mg./square foot. The quadrimaculatus males were so damaged that they did not inseminate the females well and 99% of the eggs produced from matings with virgin untreated females were nonviable. Aedes aegypti showed an average uptake of 2.5 µg./ insect, which sterilized but apparently did not impair male mating activity. Feeding for 3 days on treated food (1% of the chemical in 20% honey solution) sterilized quadrimaculatus males and caused a high degree of sterility in aegypti males. At the end of the 3-day feeding period the amount of tagged material, expressed as µg.equivalents of P32 metepa was 3.0 and 1.7 males of quadrimaculatus and aegypti. Exposure of larvae from the third instar through pupation in water treated at 10 p.p.m. failed to sterilize quadrimaculatus or aegypti significantly and the uptake of metepa was low in comparison with the other types of treatments.

Studies in Oregon showed that P^{32} metepa was rapidly absorbed and metabolized by <u>Culex tarsalis</u>. Almost complete degradation occurred in 2^{l_1} hours with phosphoric acid being the major metabolic product.

2. <u>House Fly</u>. In Oregon, certain synergists have greatly increased the toxicity of malathion to resistant strains of house flies. The synergists, simple tris-substituted derivatives of phosphoric acid,

completely overcame high levels of resistance when used at 1:1 or higher ratios of synergist to insecticide. The ability of several of these materials to synergize malathion against resistant house flies was directly related to the inhibitory effect on aliesterase activity. Effectiveness of the synergists for malathion was directly related to their ability to inhibit the aliesterase. The synergists may actually inhibit the mutant aliesterase present in organophosphateresistant fly strains.

House flies treated with the synergist tributyl phosphorotrithioate and then treated with either parathion or paraoxon accumulated greater quantities of paraoxon than did flies treated with the toxicants only. This was true with both suscpetible and parathion-resistant strains of flies. Measurements of the inhibition of thoracic cholinesterase activity provided a far better picture of the toxic action of organophosphates than measurements of head cholinesterase.

In Oregon selection of a house fly colony with Isolan produced a strain with 3-fold resistance in 14 generations. At the same time, levels of aliesterase activity declined to 40% of the original level in flies of the selected strain. This same phenomenon occurs when house flies are selected with organophosphates, indicating that the same mechanism may be responsible for resistance to both classes of insecticides.

Studies in Oregon indicated that irradiation of resistant flies with 1,000 r did not alter their susceptibility to insecticides or esterase activity. Irradiated females mated with normal males produced normal numbers of eggs but only 45% were fertile. Further studies were conducted with normal and parathion-resistant flies that had been irradiated as pupae with 600 r for seven generations. Only about 25% of the eggs hatched from matings of irradiated males and females of either colony. No changes in either esterase activity or insecticide susceptibility were apparent.

Studies in Florida and Oregon showed that a P³²-labeled chemosterilant, metepa, was rapidly absorbed, distributed, and metabolized by house flies and mice.

- 3. <u>Cockroaches</u>. A color variation which may be useful in studies of mating behavior has been isolated in a laboratory strain of German cockroaches. Breeding experiments have shown the distinctive color pattern to be a monofactorial recessive, and a homozygous strain has been isolated.
- 4. Eye Gnats. Studies were continued in Florida on the biology of Hippelates eye gnats. The laboratory colony of Hippelates pusio has

now been maintained for over 3 years. Development time (egg to adult) ranges from about 20 days in December, January, and February to a minimum of about 13 days in July, August, and September. In the laboratory, female H. pusio have laid an average of about 24 eggs each over a period of h days, with sand as an oviposition medium. Females do not mate until at least h hours after emergence. Males inseminated from 6-14 females each, over periods ranging up to 10 days, and were capable of inseminating as many as four females in 1 day. When the adults were maintained in darkness, females laid about as many eggs as they did under ordinary lighting, but egg hatch was low, indicating mating was reduced in the dark. The anatomy of the reproductive system of male and female H. pusio has been defined as a prelude to further work with sterility as a means of control.

B. Insecticidal and Sanitation Control

1. Mosquitoes. Research was continued by the Orlando, Fla., and Corvallis, Oreg., laboratories to develop more effective insecticides and other materials for controlling mosquitoes. In Florida, of over 500 new compounds screened for toxicity to Anopheles quadrimaculatus larvae, five were outstanding in their effectiveness. Of seven selected compounds screened against Culex tarsalis in Oregon, three were outstanding.

Studies were continued in Florida to find more effective residual insecticides for use in the world-wide campaign against malaria and other mosquito-borne diseases. Of 157 selected compounds screened against female adults of Anopheles quadrimaculatus, 17 were very promising, causing 90 to 100% kill of exposed quadrimaculatus for 24 weeks. Four of the compounds, Bayer 39007, Hercules 7522 C, Bayer 34098 and Bayer 41831, retained their effectiveness on sprayed surfaces for one year. In tests with treatments that had aged for 2 years, Sevin and Hercules AC-5727 plus piperonyl butoxide caused 85% kills of quadrimaculatus within 24 hours after a 1-hour exposure to 100 mg./square foot, whereas malathion and unsynergized AC-5727 were ineffective.

In Florida tests in the laboratory and field were continued to develop more effective materials for area mosquito control. Of 151 selected compounds tested as space sprays against colonized Aedes taeniorhynchus, the following four were better than the malathion standard: Kenya Oleo Resin, Bayer 34042, Bayer 30749, and Hercules 7522 C. In aerial spray tests against natural populations of salt-marsh mosquito adults, 0.05 pound per acre of Dibrom or DDVP was about as effective as 0.1 pound of malathion per acre. These sprays reduced mosquito abundance more than 90% in 6 hours.

In Oregon screening tests were conducted with a number of new insecticides against larvae and adults of <u>Culex tarsalis</u>. Three of the materials gave 100% kills of larvae at a concentration of only 0.025 p.p.m. In spray tests seven materials were 2-9 times more toxic to adult mosquitoes than malathion (standard) and the most toxic one, Bayer 39007, compared favorably with parathion.

Studies were continued on the status of insecticide resistance in mosquitoes. In Florida larvae of a strain of DDT-resistant Anopheles quadrimaculatus received from the Army Environmental Health Laboratory were about 30-40 times as resistant to DDT at the LC-50 level as the regular colony and more than 50 times as resistant at the LC-90 level. Both this colony and the regular colony were highly resistant to dieldrin, the AEHA-Orlando colony slightly more so than the regular colony. In Oregon, DDT-resistant Culex tarsalis larvae were also resistant to closely related compounds, but not to dieldrin or lindane. Further spread of resistance in Culex mosquitoes in Oregon was indicated when Culex peus showed some resistance to malathion.

2. House Fly. Research was continued at Orlando, Fla., to develop more effective insecticides and other methods and materials for the control of house flies. Approximately 150 new compounds were screened for residual effectiveness against house flies. Twenty-four materials were 93-100% effective for at least 24 weeks as compared with 12-16 weeks for malathion (standard). These materials included Thiodan, General Chemical 3583, Upjohn TUC U-12927 (plus synergist), Shell SD-4402, Bayer compounds 29952, 30237, 30468, and 29492, Hooker HRS-1422, Geigy G-27365, Stauffer N-2404, heptachlor epoxide, and twelve confidential compounds. Bayer 29952, Bayer 30237 and seven confidential compounds were still 100% effective after 44-48 weeks and are c nsidered unusually promising for controlling flies. A number of promising new materials were evaluated as space sprays against susceptible and resistant strains of house flies. Many of the materials were superior to malathion (standard) against susceptible flies but only one, Bayer 30911, was superior against resistant strains.

Over 50 selected compounds were evaluated as bait toxicants against resistant and normal strains of house flies. Eight of the compounds were equal or superior to Dipterex (standard) against both strains of flies, namely, Bayer 30237, Bayer 30544, General Chemical 6506, Stauffer N-2230, Stauffer N-2404, dimethoate and two confidential compounds. All of the compounds tested were more effective against normal than against resistant flies.

Several materials which increased the effectiveness of malathion in laboratory tests were evaluated as residual treatments in barns against sizable populations of flies. Combinations of several synergists and malathion alone were equally ineffective in these tests.

3. <u>Cockroaches</u>. Research was continued at Orlando, Fla., to develop new insecticides and more effective materials and methods for controlling cockroaches. Of 60 new compounds screened as contact sprays, 13 gave 100% mortality in 24 hours at a concentration of 0.5%. All were superior to the chlordane (standard) in speed of knockdown.

Of 10 new compounds screened for residual effectiveness against normal German roaches, the most effective was Velsicol 57-CS-47, which produced 35-100% mortality for 32 weeks. Heptachlor epoxide was effective for 14 weeks, whereas heptachlor failed after 1 week and chlordane after 2 weeks. In tests to control German cockroaches in homes, two combination dust and spray treatments were highly effective. A 0.5% Diazinon spray, followed by 2.0% Diazinon dust, and a 2.0% malathion spray, followed by 2.0% Diazinon dust, completely eliminated the roaches for 150 days, at which time the test was terminated.

Studies were continued on the phenomenon of resistance in cockroaches. After 14 generations of exposure to Diazinon, German roaches did not exhibit increased resistance to this insecticide. The eleventh generation of German roaches exposed to Baytex showed no increase in resistance. Male roaches from the Baytex, Diazinon, and "C" (chlordane-resistant) colonies showed no resistance to malathion. Roaches from the 'M' (malathion-resistant) colony and Diazinon colonies showed no resistance to chlordane. The F22, F23, and F24 generations of the "A" German roach colony (exposed each generation to pyrethrins) exhibited 7- to 36-fold resistance to activated pyrethrins when compared with the normal colony. In the 25th, 26th, and 27th generations resistance was 12- to 17-fold at the LT-50 level and 21-fold at the LT-90 level. German roaches collected from homes in 11 different locations in Orlando were all highly resistant to chlordane. One strain exhibited 2-fold resistance to malathion, but none showed resistance to Diazinon.

4. Bed Bugs. Research in Florida on bed bugs included tests with seven insecticides and a malathion standard in laboratory tests. Dow ET-15 produced 90 to 100% kills and Bayer 25141 and Bayer 373hh, 85 to 100% kills for 12 weeks. Dieldrin and Hercules 7522 gave 100% mortalities for at least h weeks. Dimethoate fluctuated in effectiveness, with mortalities ranging from h5 to 100% for 12 weeks. DDT was ineffective after 1 week. Malathion(standard) gave 100% mortalities for h6 to 12 weeks. In tests with resistant colonies of bed bugs, an immeasurably high resistance to DDT was indicated in the colony continuously exposed to this material for 17 generations. No resistance to DDT was evidenced by the "N" colony (exposed to malathion in each generation) or the DM-A colony (exposed in alternate generations to DDT and malathion) in the h7 generation. No DDT resistance was found in the DM-C colony (exposed each generation to a combination of DDT and malathion) of the 15th generation (h7). However, the "M"

colony continued to show about 17-fold resistance to malathion and resistance to malathion was 8-fold in the DM-A and DM-C colonies, based on the LT-50's. No cross resistance to malathion was evident in the D colony.

5. Body Lice. Research was continued at Orlando, Fla., to develop more effective insecticides for the control of body lice. Of 505 compounds screened, 70 were rated Class IV and IVA in toxicity and 37 were Class IV and IVA in speed of knockdown. Outstanding compounds that were rated Class IVA in toxicity and knockdown were Shell SD-1996, General Chemical 6506, General Chemical 6363, Upjohn TUC-12927, Shell SD-7169, Stauffer B-8760, Geigy 35231, Zectran, Stauffer R-3244, Hercules 7522, Bayer 44646, Monsanto CP-40273, and American Cyanamid CL-38064. Of a total of 55 compounds tested as synergists for pyrethrins, none was as effective as sulfoxide (standard). Of 30 promising toxicants tested as 1% powders, only 5, Stauffer B-8760, Zectran, Upjohn TUC-12927, Hercules 7522, and Hooker HRS-1422, were more effective than DDT and lindane. Four of the five compounds were 90-100% effective for more than 3 months. Patch tests of 29 pyrethrum and allethrin powders stored in 2-ounce cans for over 8 years indicated a slight loss in effectiveness in some but all gave 85-100% kills in 24 hours.

Extensive patch and sleeve tests were conducted to evaluate a number of powder formulations of Sevin against lice. In patch tests powders containing 1-3% Sevin and 2-10% sulfoxide were 95-100% effective for 56 days. The 1% Seven plus 10% sulfoxide powder still gave 100% kills of lice after 56 days. In similar tests powders containing 0.25-1.0% malathion were effective over 3 months, but 1% ronnel was effective only 14 days. In sleeve tests on human subjects powders containing 1% Sevin and 10% sulfoxide and 2% of each gave 100% kill of lice for more than 28 days; powders containing 3-5% Sevin alone gave 99-100% for 10 days but lost effectiveness steadily thereafter. Sleeves treated with powders containing 1 or 2% sulfoxide and 2, 3, 4, or 5% Sevin were effective for 10 to 29+ days. On the basis of cost (12¢/pound) and effectiveness, a powder containing 2% each of Sevin and sulfoxide would seem to have promise for practical use. Powders of 10% Dri-Die and 90% pyrophyllite and 100% Dri-Die were effective initially but not after 3 days.

Studies were continued in Florida on the development of resistant strains of lice and their suscpetibility to various insecticides. A normal and II-resistant strains of lice were used in these tests. Lice of the Korean A colony (under DDT pressure for 131 generations) were immune to DDT and slightly resistant to synergized pyrethrins but were equally or more susceptible than the normal colony to pyrethrins alone, lindane, malathion, and Sevin. The Freetown A colony

(under lindane pressure for 60 generations) showed extremely high resistance to lindane, moderate to high resistance to DDT, and slight resistance to synergized pyrethrins and malathion, but was slightly more susceptible than normal lice to Sevin and pyrethrins alone. The Freetown B lice (selected with malathion for 24 generations) were moderately to highly resistant to DDT and lindane, slightly resistant to synergized pyrethrins, and normally susceptible to malathion and Sevin. The Freetown C strain (selected with Sevin for 14-17 generations) was highly resistant to DDT, extremely resistant to lindane, slightly resistant to synergized pyrethrins and Sevin, and normally susceptible to malathion. Lice of the Freetown D colony (strain of Freetown A not selected for 17 generations) were extremely resistant to lindane, moderately resistant to DDT, slightly resistant to synergized pyrthrins and Sevin, and normally susceptible to malathion. The Pratt colony (selected with lindane for 65 generations) showed extreme resistance to lindane, moderate resistance to DDT. slight resistance to synergized pyrehtrins and malathion, and no resistance to Sevin. Lice of the Mason colony (selected with synergized pyrethrins for 65 generations) were 6-17 times more resistant to synergized pyrethrins than normal lice, extremely resistant to DDT, slightly resistant to lindane, and equally or more susceptible to malathion and Sevin. Three additional composite colonies (selected with DDT or malathion and not selected) were started during the year but have not been under way long enough yet to show any distinct trends in the development of resistance or loss of resistance to various insecticides.

6. Mites, Ticks, and Fleas. In Florida research was limited to the treatment of several homes for control of the brown dog tick, Rhipicephalus sanquineus. Treatments with 3% Entex and 0.5% Diazinon produced 93-100% control of the tick for at least 12 weeks.

C. Biological Control

Efforts to find pathogens and other biological agents for controlling house flies, mosquitoes, and cockroaches have been unsuccessful to date. Releases in Florida of sterile male <u>Anopheles quadrimaculatus</u> for control or eradication of wild populations has also proved ineffective in two experiments.

D. Insect Sterility, Attractants, and Other New Approaches to Control

l. Mosquitoes. In Florida extensive studies were conducted with chemosterilants, especially apholate, tepa, and metepa. In laboratory tests sterility was induced in Anopheles quadrimaculatus and Aedes aegypti males and females with these materials by feeding in adult food (honey-water), by allowing adults to walk on deposits on glass or masonite surfaces (tarsal contact) or by exposing larvae

to the materials in water solutions. Apholate did not sterilize by tarsal contact and metepa did not sterilize when larvae were exposed to it. A residual deposit of tepa sterilized males 0 to 5 days old from the laboratory colony, females 0 to 1 day old from the laboratory colony and wild-collected females that had mated, had a blood meal, and developed one to five batches of eggs. In general, depending on the dosage, treated females either showed stunted ovaries and laid no eggs or laid eggs which did not hatch. When treated males were mated to untreated females, the females received motile sperm and laid normal numbers of eggs which did not hatch. In some cases with either treated males or females, an occasional egg hatched. Most of the eggs laid by females mated to treated males showed little or no organized development. Some eggs embryonated normally, but did not hatch.

Artificial resting stations (boxes) for Anopheles quadrimaculatus were established in a breeding area in the field and treated with tepa. The deposits of the chemical were effective in sterilizing wild females that rested on them. (Males could not be collected for study.) However, the deposits remained effective for only a few days. No sterility was found in females collected outside the treated boxes, indicating that this method did not affect a significant part of the total population. Releases of gamma-sterilized or chemosterilized Anopheles quadrimaculatus males into natural populations has been ineffective to date in influencing wild populations.

In laboratory tests in Oregon, sterility was caused in <u>Culex tarsalis</u> by spraying adults with tepa, feeding adults apholate in sugar baits, and by exposing larvae or pupae to apholate or tepa in water. The amount of sterility induced was not always complete except with the sprays, which sterilized mosquitoes up to six days old. Partial sterility was also caused in <u>Aedes aegypti</u> females allowed to feed on mice treated with tepa. Preliminary tests with an olfactometer failed to show the presence of a chemical sex attractant in <u>Culex pipiens quinquefasciatus</u>, <u>Culex tarsalis</u>, or <u>Aedes aegypti</u>.

In Florida of the 400 compounds screened as mosquito repellents, 20 showed sufficient promise for further testing. In repellent tests with <u>Aedes aeygpti</u> a special formulation of dimethyl phthalate was as effective and a pressurized foam of deet was less effective than ethanol solutions of these compounds. Of 32 compounds screened for effectiveness as clothing treatments against mosquitoes one was very promising.

2. House Fly. At Corvallis, Oreg., dipping 3-day-old house fly pupae for one hour in tepa solution caused partial sterility only at high concentrations.

The remainder of the research to find materials that would induce sterility or otherwise adversely affect the growth and development of the house fly was conducted at Orlando, Fla. Of 1,263 compounds screened in the adult food, 21 caused sterility in house flies and 65 caused toxicity. Of 796 compounds screened in the larval media, nearly one-third were toxic to larvae at one or more test dosages, 13 were pupicidal, and one caused a decrease in egg viability. In secondary tests with 59 compounds that had shown promise in screening tests, 27 materials caused sterility (no oviposition or no hatch) at concentrations of 1% or lower.

Special tests were run with 24 promising chemosterilants in adult fly food to ascertain their effects on each sex. Morzid caused sterility in both sexes. Metepa and three confidential materials caused complete sterility in males but not always in females. Morzid, 5-fluorouracil and an aziridinyl compound were effective when fed to both sexes but only morzid was effective when fed to one sex. Other materials tested were less effective.

Tests were also run to compare the sterilizing action of 50 promising chemosterilants applied as larval dips, topically to adults and in the adult food. None of the materials caused sterility as larval dips, but in the adult food, metepa and 5 confidential materials caused complete or almost complete sterility. Two other compounds were effective only as topical applications.

Investigations were conducted to learn more about the action of several effective chemosterilants on house fly sexual development, mating and reproduction. In one series of tests, males sterilized by feeding 3 days on apholate proved fully competitive with normal males when placed with normal females. When only treated males were placed with normal females all eggs were sterile and 12.5% were sterile when only normal males were present. When normal females, normal males, and treated males were combined at ratios of 1:1:1 and 1:1:2, 65 and 80% of the eggs were sterile and higher ratios of 1:1:3, 1:1:5, and 1:1:10 resulted in 99.9-100% sterility. Additional tests at these ratios confirmed that actual sterility was higher than the expected. Males given food containing 0.4-1.0% apholate for 3 days were sterilized for life but lower concentrations of 0.1-0.3% were not 100% effective. Exposures of males on residues of 500-1000 mg./kg. of tepa on plywood panels caused only partial sterility (12-72%) but when applied at 250-500 mg./square foot with sugar, tepa and apholate produced 91-100 and 99-100% sterility, respectively. When applied in glass jars, residues of 250 mg./square foot of tepa or metepa completely sterilized flies in 2- to 4-hour exposures for 30 days but not for 60. Deposits of 100 and 50 mg./ square foot caused complete sterility for 14-30 and 14 days, respectively, but deposits of 10-25 mg. were mostly ineffective. Baits containing 0.5% of tepa or metepa effectively sterilized flies after

aging 30-37 days on most types of surfaces. Some loss in effectiveness in tepa was apparent on metal and masonite and in metepa on wood, but none was apparent on painted wood, asphalt, metal or wax paper.

Adult blow flies, <u>Phaenicia cuprina</u>, were rendered sterile after feeding on ground meat containing 0.5-1.0% apholate or 0.25-0.5% metepa. The flies oviposited, but few or no eggs hatched and all larvae failed to develop.

Cytological studies showed that the ovaries in normal 3-day-old flies were 6-8 times as large as those in females fed for 3 days on 1.0% apholate. After feeding ceased some growth occurred but ovaries never attained normal size. Females mated to males fed on 0.4-1.0% apholate oviposited but the eggs showed little or no embryonic development, whereas females mated with males fed 0.1-0.3% laid some viable eggs and some without embryonic development. Similar results were obtained in tests with normal and insecticide-resistant strains of flies. Apholate at 1% in adult food over a period of 24 hours inhibited but did not eliminate ovarian development in females. The greatest effect was noted at 72 hours after eclosion in the nurse cells in the first and second egg chambers. The chromatin was irregular and nuclei had bizarre shapes. Oocytes matured in the first cell but not in the others. The germarium was also affected as the third egg chamber was not visible until 168-192 hours after eclosion compared with 96 hours in normal flies.

Weekly applications of cornmeal bait containing 0.5% tepa on an isolated refuse dump reduced adult house fly populations from 47 to 0 in 4 weeks and counts remained at 0 as long as the bait was distributed. The viability of eggs of female flies declined from 11 to 10% in 4 weeks and to 1% in 5 weeks. After baiting ceased, populations increased slowly but the percent viability of eggs was normal after 2 weeks. Additional small-scale field tests with cornmeal-chemosterilant baits against flies were conducted on a small garbage dump and in a poultry house. Weekly applications of apholate baits on the dump and of metepa baits in the poultry house caused some sterility and reduction of fly populations. Applications 5 days a week resulted in a high degree of control and high sterility in flies in both areas. Sterility among females was slightly higher than in males.

Irradiation caused greater damage to young (2- to 4-day-old) house fly pupae than to middle-age pupae. Virtually no damage was apparent in old pupae and longevity of the adults from them was greater than those from younger pupae. Irradiation of pupae 0-36 hours before adult emergence did not produce complete sterility and there was some slight recovery as second matings resulted in an increase from one to four progeny per female. No recovery occurred in pupae irradiated 72-96 hours before adult emergence.

There were slight differences in the competitive ability of male flies given 1% apholate in food and those irradiated with 2850 r. Neither radiation nor the apholate completely sterilized the males but at a 4:1:1 ratio, chemosterilized males caused a reduction in egg hatch of 81.4% compared with 78% for the irradiated males.

Research initiated at Orlando, Fla., on the development of physical and mechanical methods of controlling flies and other pests with particular emphasis on radiant energy, was continued at Beltsville, Md. Preliminary tests were necessary to develop suitable techniques. In comparative tests a 15-watt BLB fluorescent light was much more attractive to flies than 15-watt daylight and BL fluorescent lamps, but in other tests there was not much difference in the attractiveness of 4-, 8-, and 15-watt BLB, BL, and daylight fluorescent lamps. Various combinations of these lights attracted about equal numbers of flies. There was no significant difference in the light attraction of 1-, 2-, and 6-day old flies with the 15-watt BL fluorescent lamp; however, flies 3 days old were more attracted to the light if they were virgin males or females, than if they had mated.

- Cockroaches. Research was initiated in Florida on chemicals affecting life processes in cockroaches. Several compounds that had been effective as chemosterilants of other insects also caused sterility in cockroaches when administered in the nymphal diet. Metepa at a concentration of 0.5% in the food caused sterility when treated males and females were crossed but this concentration was only partially effective against males and ineffective against females. When both sexes of cockroaches were fed methiotepa no egg hatch occurred. However, high toxicity of the chemical to the roaches precluded further studies that would have been needed to determine whether one or both sexes were sterilized. Male roaches treated with 0.1% tepa were sterilized; a 0.25% concentration sterilized both sexes. Studies with specimens treated with 1.0% apholate indicated that the males were sterilized by this concentration, but a higher concentration will be necessary to sterilize females. Aphomide at 1% deformed some of the egg capsules and reduced the egg hatch, but failed to sterilize either sex completely. Methiotepa, tepa, and aphomide delayed development of nymphs at some concentrations, and several other compounds proved toxic to the roaches.
- 4. <u>Body Lice</u>. At the Orlando, Fla., laboratory, lice fed on rabbits treated with various doses of a chemosterilant, aphoxide, lived and reproduced normally indicating that the material was not effective. A male rabbit receiving the highest dosage was mated to a normal female and five young resulted, indicating the material did not affect his fertility.

Tests were conducted to evaluate the effectiveness of 70 systemic insecticides against body lice when the materials were fed to rabbits and lice fed on the rabbits. One material, pivalyl at 100 mg./kg. continued to kill lice for 7 days. Other compounds which caused 100% kill of lice for 1-5 hours or at 1 or more test periods without being toxic to the rabbits were: American Cyanamid CY-12503, 200 mg./kg.; American Cyanamid CL-28865, 5 mg./k.g; Bayer 25198, 10 mg./kg.; American Cyanamid CL-26691, Baytex and Shell SD-3562, 25 mg./kg.; Bayer 34727 and Monsanto CP-10502, 50 mg./kg.; and Bayer 37342, 100 mg./kg. Special tests with rabbits treated with Butazolidin (20-25 mg./kg.) showed that this material was effective for longer periods against first and second than against third instar lice. Female lice were somewhat more susceptible than the males.

5. Mites, Ticks, and Fleas. Research was continued in Florida on the development of repellents and other methods for protecting humans from mites, ticks, and fleas. Three hundred seventy-six compounds were screened for repellency to nymphal lone star ticks, 61 of which were rated Class IV. The outstanding material (confidential) was 95-100% effective for 77-84 days. Another confidential material gave 95% repellency for 49-77 days. Clothing treated with these two effective materials (confidential) withstood four and three washes, respectively, as compared with three washes for benzyl benzoate (standard). Other materials were effective for 24-49 days. Only two of 354 compounds tested were rated Class IV against chigger mites.

Screening tests against fleas were conducted with a backlog of 133 compounds that had been rated Class IV in repellency to ticks. Fourteen materials were effective for 15 days or more. The outstanding materials and duration of their effectiveness were as follows: \underline{m} -Chloro- $\underline{N},\underline{N}$ -diethylbenzamide, 57 days; oxime butyrophenone, 51 days; and heptyl mandelate, 50 days. Several series of pen tests were conducted to compare the effectiveness of a number of promising repellents and deet (standard). In these tests \underline{N} -butyryl-1,2,3,4-tetrahydro-quinoline was slightly less effective than deet for 2 weeks but more effective after 4-5 weeks. Both were more effective than other test materials. In other pen tests combinations of deet and \underline{N} -butyryl-1,2,3,4-tetrahydroquinoline and the latter alone are more effective than \underline{N} -butyryl-1,2,3,4-tetrahydroquinoline and the latter alone are more effective than \underline{N} -butyryl-1,2,3,4-tetrahydroquinoline and the latter alone are more effective than \underline{N} -butyryl-1,2,3,4-tetrahydroquinoline and the latter alone are more effective

6. Eye Gnats. Both males and females of <u>Hippelates pusio</u> were sterilized in laboratory tests in Florida when aphoxide was administered in the adult food or as a residual deposit on glass surfaces. When both sexes received aphoxide in their food (either 0.5% or 1%), 90-100% sterility was achieved. Exposures to 5 and 10 mg. of aphoxide per square foot on glass surfaces also caused 90-100% sterility and there was no recovery of fertility within 15 days.

Olfactometer studies indicated that male gnats were attracted to air passed over the females. Both sexes were attracted to stale fish. Liver, ham and eggs were less attractive.

E. Evaluation of Equipment for Insect Detection and Control

Field studies in Florida using light traps, small animal baited or carbon dioxide baited traps, and vehicles, along with releases of tagged or sterile laboratory insects, have shown that collections at night from parked vehicles was the best method of collecting anopheline mosquitoes either for recapture of released mosquitoes or collection of wild mosquitoes.

In cooperation with the Brevard County, Fla., Mosquito Control District, it has been determined that aerial fogging equipment using malathion as an insecticide will give effective control of mosquitoes. Twelve-hundred acres can be treated in one hour using 70 gallons of solution.

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AREA 18. BEES AND OTHER POLLINATING INSECTS

Problem. The pollination of some 50 seed and fruit crops depends on an abundance of honey bees and other pollinating insects. Although certain wild bees help to pollinate cultivated crops, honey bees are estimated to account for three-fourths of the pollination by insects. Most growers are not fully aware of the importance of honey bees and the wild bees in the production of pollinated insect crops. Therefore, research that leads to more efficient and economical production of honey bees is imperative to insure effective pollination of many crops and the economical production of honey. A problem of major significance is the increasing use of pesticides, many of which are hazardous to bees or destroy important pollen and nectar sources. There is need for more knowledge on the management of bee colonies; breeding of improved hybrid bees; physiology and behavior of queens, drones, and workers; and on the various diseases and pests of the honey bee and means for their control. There is also need to study the many facets of the complex pollination problem to integrate effectively populations of honey bees and other pollinating insects with crop needs and practices. More knowledge should be obtained about wild insect pollinators and their management. is also essential to study the effects of farm practices, such as the use of different pesticides, changes in crops, soil management, and harvesting, on the economy of the beekeeping industry and the survival of pollinating insects, and to develop procedures to minimize losses from such practices. Information is needed on nectar and pollen plants for use in conservation program efforts to provide bee forage areas in wastelands, watersheds, and roadsides. The nutrition of bees and the nutritive value of different pollens to bees require intensive investigation together with basic nutrition studies for development of pollen substitutes.

USDA PROGRAM

The <u>Department</u> has a continuing program involving apiculturists, geneticists, microbiologists, physiologists, and entomologists, engaged in basic studies and in research concerned with the application of known principles to the solution of crops pollination problems for the farmer and problems that affect the beekeeper. <u>Bee breeding</u> investigations at Baton Rouge, La., are cooperative with the State Experiment Stations of Louisiana, California, and Wisconsin, the Ohio Honey Bee Improvement Cooperative Association, and the Ontario Agricultural College, Guelph, Ontario, Canada. <u>Bee management</u> investigations at Madison, Wis., are cooperative with the Wisconsin and Arizona Experiment Stations, the Ohio Honey Bee Improvement Cooperative Association, the Department of Apiculture at Ontario Agricultural College, Canada, the Abbott and Pfizer Laboratories, the Eastern Utilization Research and Development Division,

and the Agricultural Engineering Research Division. Investigations on <u>bee diseases</u> are carried on at Beltsville, Md., and Laramie, Wyo., in cooperation with the Louisiana, Wisconsin, and Wyoming Experiment Stations. <u>Honey bee pollination</u> investigations at Tucson, Ariz., are carried on in cooperation with the Experiment Stations of Arizona, California, Louisiana, Utah and Wisconsin. <u>Wild bee pollination</u> investigations at Logan, Utah, are conducted in cooperation with the Experiment Stations of Arizona, Utah, Louisiana, Wyoming, Idaho, Oregon, Washington, and the Crops Research Division, Agricultural Engineering Research Division, and private beekeepers and farmers.

The <u>Federal</u> scientific effort devoted to apicultural research totals 22.1 professional man-years. Of this number 6.7 is devoted to breeding and management to improve productivity in honey bees; 4.7 to biology and control of diseases and pests of honey bees; 5.5 to behavior and utilization of honey bees and other insects in crop pollination; 2.7 to effect of pesticides and other farm practices on honey bees and other pollinating insects; 0.5 to chemical residues in honey; and 2.0 to program leadership.

P. L. 480 grants total 9.25 man-years. Bee breeding research is being conducted under P. L. 480 funds at the Central Apicultural College, Warsaw, Poland (2.5 man-years) and at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Rio Claro, Sao Paulo, Brazil (3 man-years). Bee disease research under P. L. 480 funds is underway with the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (3 man-years) and with the Instituto Nazionale di Apicoltura, Bologna, Italy (3/4 man-year).

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 14.3 professional man-years divided among subheadings as follows: Biology and control of diseases and pests of honey bees 2.3; breeding and management to improve productivity in honey bees 3.4; behavior and utilization of honey bees and other insects in crop pollination 6.4; effects of pesticides and other farm practices on honey bees and other pollinating insects 1.9; and chemical residues in honey 0.3. Iowa, New Hampshire, Massachusetts, Mississippi and California are conducting studies on bee diseases including antibiotics, genetics of disease resistance, practicability of vaccine treatments for foulbrood and the mechanism of food transfer from adult to larvae. lowa, New Hampshire, Massachusetts, Texas, and California are conducting studies of honey plants, food materials in pollen, management practices, nutrition of bees, storage of pollen, processing of honey, queen rearing and the use of electricity in beekeeping operations. Minnesota, New York, Texas, Washington, and Oregon are conducting studies on pollination of alfalfa, apples, cranberries, vegetable and other crops for seeds; the effects of controlled

burning of pine areas on the survival of native pollinating insects; biology and distribution of native bees and methods of protecting them; distance between alfalfa varieties to prevent crossing; and improvement and artificial establishment of alkali bee nesting sites. Massachusetts, Texas, California and Washington are carrying on studies of bee losses and how to avoid them, toxicity of pesticides to bees, and the effects of insecticide treatments on honey production. California and Washington are conducting studies on determinations of pesticide residues in honey.

Beekeeping industry organizations. Several organizations in the beekeeping industry are engaged in developing means of utilizing the products of the honey bee, particularly honey and royal jelly. Some of these organizations work closely with growers and packers of frozen fruits and similar products. One bee supply company is developing and marketing hybrid bees. Estimated annual expenditures of these organizations are equivalent to approximately 2 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Diseases and Pests of Honey Bees

- I. <u>Diagnostic Service</u>. At Beltsville, Md., 511 samples were diagnosed for diseases during 1961, of which 315 were for brood diseases and 196 for adult diseases. A sample of comb submitted from Panama was infected with American foulbrood. Six samples from Argentina and one from Brazil were infected with European foulbrood. One sample from Argentina was infected with parafoulbrood.
- 2. Acarine Mites. The survey of honey bee inhabiting mites was continued at Beltsville but no disease-causing internal mites, Acarapis woodi, have been found. External mites of the genus Acarapis were present on bees from Florida, Georgia, Louisiana, Maryland, North Carolina, and Wyoming. In Florida a single apiary was infested by Acarapis dorsalis and II out of I2 apiaries by A. externus. Wyoming about 60% of the overwintered colonies and 3% of the bees in them were infested by A. dorsalis in February and March. In package bees shipped from Louisiana 2% of the bees were infested by A. vaqans and 8% by A. dorsalis. Package bees from Georgia showed 1% of the bees infested by A. externus and 7% by A. dorsalis. All stages eggs, immature stages and adults - of both sexes of all 3 species were still present in December. The effect of external mites on the colonies is considered inconsequential. Attempts to culture external mites of the honey bee under laboratory conditions on bee hemolymph and artificial media were unproductive.

Study of the honey bee mites of the genus <u>Acarapis</u> at Beltsville, Md., has revealed a second character in the female adult that may be useful in differentiating the various species. This character is a longitudinal median apodeme located between the first two pair of legs, the portion of the body described by Vitzthum as the propodosoma. In <u>A. dorsalis</u> this apodeme extends the full length of the propodosoma. In <u>A. woodi</u>, <u>A. externus</u>, and <u>A. vagans</u> it extends only about two thirds the length of the propodosoma.

- 3. Acarine Disease. As the result of a visit to various European laboratories the following information was obtained on Acarine disease, a serious disease of bees not present in the United States. Acarine disease caused by the internal mite A. woodi is considered a major problem particularly in Italy, France, and Switzerland. There is general agreement in Europe that Folbex fumigation is at present the most effective treatment for Acarine disease. This treatment is being used in Italy, France, Switzerland, West Germany and England. The fact that Acarine disease is ordinarily recognized only after it is well established and that a single acarine mite in a single bee from a single hive can reestablish an infestation presents difficulties for chemical control of this disease. Present methods of chemical control are complicated and require much time for application.
- 4. <u>Wax Moth</u>. Early results of observations and tests at Baton Rouge, La., with the greater wax moth, <u>Galleria mellonella</u>, and bumble bee cells indicate that wax moths can sometimes develop in bumble bee cells. Thus, there is a possibility that bumble bee nests might serve as hosts of the wax moth.
- 5. Nosema Disease. This disease caused by the protozoan parasite, Nosema apis, is of great concern to the receivers of package bees and queens in the Northern States and Canada. When queens become infested they are superseded, causing great economic loss to the honey producer. Infested worker bees have a shorter than normal life span but the effect on production is less well understood by the beekeeper and more difficult to demonstrate than supersedence of the queen.

In a survey of 42 apiaries in Maryland IO, or 25% were infested with Nosema in detectable amounts.

Ten bee samples of worker honey bees were taken from colonies in the Baton Rouge, La., area during the season and examined for the presence of Nosema. The peak of infection was in April, May, and June; a reduction occurred in August to November; and little or no infection was present in January and February. Colonies headed by

inbred queens were heavily infected (from 10 to 90%) during the year except in January, February and December. Honey production colonies had more infected bees over a longer part of the season in 1961 than in 1960.

At Laramie, Wyo., aqueous suspensions of <u>Nosema apis</u> spores treated with ultraviolet light for I hour still produced infection in caged bees, but the same suspension treated for 5 hours produced no infection among sister bees in four cages.

In several series of tests at Laramie, bees from colonies showing original infections of 5-30% Nosema disease, when placed in cages and fed plain sugar syrup, increased their infections to 82-90% by time of death. However, sister bees fed Nosema spore suspensions treated with ultraviolet light for 5 hours developed only 8-33% infections at death, suggesting that feeding such killed or attenuated spores may aid the bees to develop some protective mechanism against further infection. Temperatures of the suspensions treated with ultraviolet light attained $122-125^{\circ}$ F. in one case, and $133-138^{\circ}$ F. in another. A similar suspension treated with heat in an oven at $135-140^{\circ}$ F. for 5 hours, when fed to caged bees, resulted in their increasing original Nosema infections of 0-30% to 27-78% at death, compared with 74-90% infections among sister bees fed plain sugar syrup.

A survey for Nosema disease in Wyoming in all colonies showed that two apiaries apparently remained free of Nosema throughout the year, but infections were severe in the spring in one apiary, and variable in other apiaries in the spring and autumn.

At Madison, Wis., Nosema infections were determined at monthly intervals in overwintered colonies, package bees, attendant bees for queens received, and all queens that died in storage or were recovered from colonies. Only 6% of 273 test queens received from Pelee Island showed infected attendant bees, a low level, compared with the usual 30 to 40% in previous years. Fumidil B was fed to the nuclei on the island to control Nosema.

Nosema infections were present in 39% of 323 queens lost in a holding colony at Madison. These queens included those stored as virgins following insemination and queens that had laid in nuclei or colonies during the summer. All storage colonies are being fed 100 mg. of fumagillin in syrup one month prior to their use and 25 mg. at weekly intervals while they are used for queen storage in an attempt to reduce these losses. One-hundred queen nuclei fed one-half cup of fumagillin syrup (100 mg./gal.) at weekly intervals showed very low

infection (19%) compared with the normal expectancy of 10 to 75%.

Nosema disease causes serious losses in bee management, especially in the production, shipment, and use of queens and package bee colonies. A United States — Canadian Nosema Committee was established to make recommendations for the reduction of Nosema losses based on information currently available, to develop survey procedures including evaluation of the recommendations, and to coordinate future Nosema research in both countries.

- 6. Amoeba Disease. At Laramie, Wyo., microscopic examinations of the ventriculi of 20 or 30 bee samples from all colonies were made monthly from January through June and again in December. Malpighamoebe mellificae cysts were found in only a single bee in one colony in December, and it was mixed with an infection of Nosema spores.
- 7. American Foulbrood. Pollen trapped from the legs of bees in colonies infected with Bacillus larvae, cause of American foulbrood, at Baton Rouge, La., was fed to 12 package colonies installed on newly drawn combs at Laramie, Wyo. All of the colonies fed this pollen and gorged with plain sugar syrup became diseased; therefore, beecollected pollen (as well as honey) from diseased colonies is a dangerous source of infection. Among colonies similarly fed this pollen but gorged with sugar syrup containing sodium sulphathiazole, 1 of 6 colonies fed pollen as the only source of infection, and 4 of 6 fed the pollen plus American foulbrood "scales," eventually became diseased, but not until after the sulfa gorgings had been discontinued. Colonies fed American foulbrood scales mixed with pollen developed the disease more quickly and to a greater extent than similar colonies fed the same amount of scales mixed in sugar syrup. This confirms the serious danger of pollen as a source of infection in American foulbrood disease.

In vitro tests conducted at Laramie of the antibacterial activity of Tylosin lactate, a water-soluble salt of Tylosin that showed unusual activity against spore formers, demonstrated inhibition of <u>Bacillus larvae</u> at a level of 0.1 p.p.m. Tylosin lactate also showed inhibition of <u>B. alvei</u> at a level of 1.0 p.p.m. and <u>B. laterosporous</u> at a level of 10.0 p.p.m. The level of activity against <u>B. larvae</u> suggests that this antibiotic may be effective for control of American foulbrood.

A study at Beltsville, Md., to determine the susceptibility of honey bee larvae to spores of <u>Bacillus larvae</u> by direct oral introduction was made by introducing spore suspensions into the midgut of 5-day-old larvae and incubating the inoculated larvae at temperatures of 25°, 30°, and 34° C. Significant mortalities occurred when the

dosages ranged from 30,000 to 3,000,000 spores per larva. This finding suggests additional study of the function of the dosage in relation to the apparent age immunity of the honey bee larvae to <u>B. larvae</u>.

8. <u>European foulbrood</u>. In work at Laramie, Wyo., either erythromycin thiocyanate or erythrocin stearate, at a dosage of I gram per quart — found promising for control of European foulbrood disease — or at 5 times this dosage, was fed to worker honey bees in cages kept at a constant temperature of 30 — 31° C. and a relative humidity of 37 — 46%. Mortality rates were almost identical in four cages and averaged about 78% of the mortality of sister bees fed plain sugar syrup. Therefore, either form of the antibiotic should be satisfactory for gorging bees in colonies for European foulbrood treatment.

Erythrocin stearate and erythrocin thiocyanate (Gallomycin) both gave good control of European foulbrood when fed as a preventive in May and eliminated the disease when definite infections were indicated. Three gorging treatments at 5-day intervals were made using 100 mg. of antibiotic in a quart of syrup poured over the bees. Weak colonies, especially those headed by inbred queens, required subsequent treatments because the bees failed to clean out all infective material. The thiocyanate salt of erythromycin showed no evidence of toxicity either to colonies or caged bees. Hence, there is no need for using the stearate formulation.

Artificial inoculations of individual brood cells with one of three dilutions of spores from agar slant cultures were made at Beltsville, Md., using three different bacterial species found as secondary invaders in European foulbrood. Inoculations with Bacillus alvei or with B. apiarius resulted in negligible removal, and only one diseased larva from which B. apiarius was recovered. Inoculations with B. laterosporus resulted in high (69-94%) removal by the nurse bees before the larvae were sealed, compared with negligible (1-4%) removal of sister larvae in alternate rows of the same brood combs. No dead larvae were found among the sealed brood.

9. Sac Brood. At Laramie, Wyo., inoculations of individual brood cells with sac brood inocula produced highly variable results. Filter paper filtrates of diseased larvae, when fresh, produced 80 - 88% infection, but the same filtrates refrigerated for 1 to 26 days produced no or negligible infections. Either the supernatant or the resuspended sediment of centrifuged filtrates failed to produce disease, while removal of larvae before sealing was sometimes negligible and at other times complete. Inoculation with Millipore filtrates caused almost complete removal of larvae before sealing, even when control larvae contaminated with sterile distilled water showed negligible removal, but no diseased larvae were found among the sealed brood.

B. Breeding and Management of Honey Bees

I. <u>Breeding</u>. At Baton Rouge, La., lines resistant and susceptible to DDT are being developed for a study of inheritance of DDT resistance. Four queens from colonies that had become increasingly resistant to DDT over the last four years were obtained from the Citrus Experiment Station, at Riverside, Calif. Queens and drones reared from the two most resistant colonies were treated with DDT and matings made between the survivors for the continuation of selection for resistance next season. Nine of the inbred lines were tested for resistance by the Riverside Station in a search for stock from which a susceptible line could be established. Results of the tests have been very variable with the topical application used and some refinement of method is needed.

In cooperation with the Ontario Agricultural College, three stocks of bees were imported in immature stages from England into the United States in order to test the practicability of this method for importing breeding stocks without the danger of introducing Acarapis woodi, a mite causing Acarine disease of adult bees. Dr. M. V. Smith, of the Canadian Institution, prepared the material at the Rothamsted Experiment Station, brought the stages over from England in a batteryheated incubator which he carried as personal luggage to Baton Rouge. where the rearing to maturity and mating was accomplished. Female material was brought as eggs, larvae, and queen pupae. Male material was brought as drone pupae, and some semen was received by mail. Immature stages have not yet been successfully shipped by mail. all three stocks mature queens were obtained from all types of female material and mature drones from the drone pupae. Artificial matings were made to secure the three stocks and mated queens and virgins of all stocks were sent to the Ontario Agricultural College and to the Madison, Wis., laboratory.

In a test at Madison, Wis., with full colonies to measure differences between inbred lines of bees only 22 colonies survived from an original 60. Early in February it was discovered that Nosema disease was widespread throughout the test colonies. Shortly thereafter European foulbrood appeared and its spread was also rapid. No medication was applied and these two diseases, for all practical test purposes, rapidly eliminated seven of the II lines under test. Survival was adequate in two lines but production in these was hampered by inadequate colony buildup. Two lines built up good populations, possibly indicating some resistance to European foulbrood, but Nosema persisted even in the better colonies of both lines.

In a study of the temper of honey bees at Baton Rouge, La., electrical shock was used. Individual bees were able to detect voltages as low as .8 volt and some bees would start stinging at I volt. Very few

would resist stinging when subjected to 2 volts. Since the differences between lines were so small and the repeatability was not consistent within lines, it was concluded that this type of shock was not an adequate tool to measure the temper of honey bees.

At Baton Rouge, the variability and inheritance of egg weight were studied in inbred lines and their hybrids. The average egg weight in samples of 15 eggs from 75 inbred queens of varying lines ranged from .1207 mg. to .2157 mg. Crosses between high and low weight lines resulted in an intermediate egg weight with no apparent heterosis. Although weight variation is largely of genetic origin, there is evidence that environmental effects contribute to the total variability. A manuscript, "Egg Weight Variability and Its Inheritance in the Honey Bee" by S. Taber, 3rd, is in press.

At Madison, Wis., the hybrid (ZX)(MI) produced 100 pounds more honey than (89)(MI) and (Z2)(MI) hybrids. The respective average yields obtained from the four lines involved in the hybrids were 288, 277, 177, and 172 pounds of honey in excess of their 12-month consumption.

Commercial beekeepers who used the (ZX)(MI) commercial hybrid through cooperation with the Ohio Honey Bee Improvement Cooperative Association rated this stock above average or superior in production (94%), brood rearing (95%), nonswarming (78%), temperament (69%), and weight of winter brood nests (80%). Use of bur comb, propolis, and supersedures were rated average by 70, 55, and 56% of the beekeepers, respectively. Excessive queen losses were indicated by several large operators who purchased early queens. There was evidence that a high incidence of Nosema infection accounted for these losses. Nosema disease is most serious early in the season. The (ZX)(MI) hybrid has proved superior in productivity and general behavior characteristics and should be released to the industry. The hybrid breeding research has been in cooperation with the Ontario Agricultural College, and the Ohio Honey Bee Improvement Cooperative Association.

Ten inbred lines have been maintained, breeder queens produced, and numerous hybrid combinations of the inbred lines made for prescreening tests to estimate their combinability and colony characteristics. Out-crossed inbred queens are difficult for commercial queen breeders to use. Preliminary tests of 3-way hybrid breeder queens (6Z)(X) to establish vigor in breeder queens (grafting mother) were initiated for testing (6ZX)(MI) with (ZX)(MI). Lines 6 and Z are of similar stock origin. In theory this should be better than back-crossing (ZX)(ZX) to obtain greater vigor in the breeder queen.

2. <u>Management</u>. The addition of 2 to 3% of royal jelly to queen candy supplied caged queens introduced for multiple queen storage to colonies for overwintering at Madison, Wis., was beneficial in securing their

initial acceptance by the colony. Once the queens are accepted, the bees feed them. Their survival depends upon having a large population of bees, protected from Nosema infection, with the position of the caged queens and food supply organized so that the winter cluster cannot pull away from the queens under severe low temperature conditions. Substantial progress has been made but certain problems have not been completely resolved to insure the desired level of success.

As a follow-up to apiary-shading studies in 1960, shading and "liberal" supering were tested in various combinations in 1961 at Logan, Utah. Liberal supering, whether in shade or in the open, had a very beneficial effect on honey production. The shading of colonies was also beneficial but to a lesser degree. The extra supering may have benefited the colonies both by its temperature buffering effect (which was measurable) and by providing extra comb space for the bees to distribute nectar for ripening.

At Logan, Utah, a large number of materials were tried as substitutes for phenol in driving bees from supers of honey. Materials that showed promise in comparison with phenol were as follows: Isopropanol, 50%; acetic acid, 25%; xylene, 50%; iso-butyric acid, 50%; acetone, 100%; anise oil; propionic acid, 80%; pine oil, 100%; pine oil, 50%; pyridine, 50%; and chloroform. Other materials tried were either too strong or too weak or had other undesirable properties.

A new, rapid method of screening compounds for repellency to honey bees in the hive was developed at Tucson, Ariz. Fumes of the candidate compound were blown upon bees on a comb, and the response noted. About 135 selected compounds were rescreened and 7 new ones were tested on colonies for their capacity to drive bees off combs. The most promising compounds were acetic acid, propionic acid, and propionic anhydride, of which the last was the most repellent. Use of these compounds does not create a residue problem in honey. A long series of tests with them was run on colonies to develop a good method for driving bees from honeycombs for harvesting honey. Owing to high volatility and a tendency to stupefy the bees on long exposure, control of dosage was the principal problem in adapting the compounds to practical use.

At Tucson, Ariz., and at Madison, Wis., intensive tests were made of new repellents and methods for removing bees from supers to replace the use of carbolic acid. Propionic anhydride was suggested after it was found that both propionic and acetic acids had good repellency under some conditions but were unsuited under the variations that exist in the field. A successful method of applying propionic anhydride

was developed and recommendation made to the industry in August 1961 (Correspondence Aid 33-16). Less than .01 p.p.m. is applied to the colony per pound of honey, and probably less than 1% of this amount comes in contact with the honey. Laboratory mice exposed under the repellent procedure showed no adverse reactions or injury. Preliminary gas chromatography analyses by the Wisconsin University Department of Biochemistry showed equivalent levels of propionates in control honey as in that removed by the new method. Best results were obtained with propionic anhydride in a special vaporizing chamber from which the fumes were blown into the honey super by an attached bellows. Numerous modifications of the apparatus were made and tried on colonies with variable results which furnished good leads for improvements.

The "bee room" in the Apiculture Research Laboratory at Logan. Utah. was tested for the first time. Colonies brought in on February 6, 1961, reacted well, collecting pollen from dishes by the next day and readily taking honey and, to a lesser extent, sugar syrup. During the first six weeks the square inches of brood increased from 5 to 603. After this a pollen-soybean flour mixture was fed and the brood began to decline. Mint oil was needed to make the syrup attractive enough for bees to start using it. It was possible to change radically the position of the pollen feeder by short successive moves. It was also possible to regulate the flight pattern of the bees by turning off banks of lights in various parts of the room. The bees were very "reluctant" to fly through shadowed areas and when in a shadowed area soon started back toward the hive. The bees worked several types of potted flowering plants very well and also individual flowers scattered on a table. When bees were trained to honey and to syrup feeders, those trained to the honey were much more faithful than those trained to the syrup.

C. Pollination by Honey Bees

I. Pollination of Cactus. Tests were conducted at Tucson, Ariz., on pollination of the organpipe cactus in 1961 similar to tests in former years on pollination of the saguaro cactus. Results were similar, that is, the organpipe cactus is self-sterile but can be cross-pollinated by bees, bats, and probably other agents. Pollination is possible almost the entire time the flower is open - from about 7:00 p.m. to 9:30 a.m. Unpollinated flowers shed in about 4 days. Pollinated ones produce edible ripe fruit in about six weeks.

When scout bees were prevented from returning to a hive in a cage after visiting the enclosed flowering saguaro and other bees did not leave the hive. This would indicate that retaining the bees in hives near pesticide-treated fields in the morning until pesticide application is completed should reduce damage to the cluster by the pesticides.

- 2. <u>Pollination of Cotton</u>. Bee visitors to cotton flowers in Arizona increased sharply after the disappearance of competing alfalfa flowers. With each 100-yards distance from the apiary the bee visitation to cotton flowers decreased significantly. This points up the importance of distributing the colonies so they are near the plants to be pollinated.
- 3. Pollination of Clovers. Sixteen colonies of honey bees were provided to pollinate from 20 to 25 acres of white clover, Trifolium repens, in an isolated field in Louisiana. Part of the field consisted of three-year old plants and part of one-year plants. Staked areas were used, at varying distances from the apiary, to provide data on number of bee visits to the areas, number of blossoms, mature heads, florets per clover head, percent fertilized florets, seeds per floret and per head, and weight of harvested seed per area. Soil analyses were made for each area the phosphorus, potassium, calcium, and magnesium content, and the pH value.

The number of honey bee visits and seed production per area could not be correlated with distances of areas from the apiary or with nutritive elements present in the soil. The average number of fertile florets per clover head was 58.5, up from 40% in 1960. During the past three years at this field pollination has improved from year to year. About one-half of the pollen collected in a pollen trap early in May was from the white clover; the other half was from Nyssa sp. Late in May it was chiefly from magnolia and Vitaceae. The low percent of fertile florets and the collection of large amounts of pollen from plants other than clover shows that the clover was not as attractive to the honey bees as desirable.

At Madison, Wis., cooperative tests with the Department of Agronomy of the Wisconsin Station were made on the pollination efficiency of a normal colony of honey bees confined under a 1-acre saran screen cage on a new selection of red clover. The colony behaved normally, provided uniform distribution of bees, gathered enough pollen to maintain brood production, but gathered little, if any, nectar. The number of foraging bees was theoretically ample to pollinate all the blossoms but the seed yield of 40 pounds per acre was disappointing. Temperature and humidity conditions were similar inside and outside the cage. The light was depressed 50% by the fine mesh screen, however, and this caused the plants to grow rank and have weak stems. Based on results obtained under similar small breeding cages, the bee population may have to be increased several times to obtain satisfactory seed set.

4. Pollination of Alfalfa. At Logan, Utah, general conclusions from 4 years of cultural studies on the alfalfa seed-production plots at the cooperative nursery at "F" field are that good seed-producing varieties will respond less to improved cultural practices than poor seed-producing varieties, and that this response will be greater in years when conditions are less favorable for seed production. Satisfactory seed yields can be obtained from Utah Syn. C without any particular attention to stand density. In other words, this variety is adapted for use for both seed and hay production on the same stand. In the case of Ranger and Lahontan, however, the response to row culture and thinning is sufficent to justify their use.

In 1960, a year that was exceptionally dry at Howell, Utah, the last irrigation that could be applied in the cooperative nursery was several weeks before the bees started collecting pollen to any extent. By this time there was little difference between the "wet" and "dry" plots. There was no difference between them in bee populations or percent pollen collectors, but tripping of the alfalfa blossoms was significantly higher on the "wet" plots. Yields were higher on the dry plots, probably because they matured earlier and had less damage from an early frost. There was a good correlation between the number of tripped flowers per raceme and the percentage of honey bees collecting pollen.

In 1961, for the first time since the nursery was started at Howell it was possible to get good differences in stand and moisture levels on plots of alfalfa at the same time that bees were collecting the pollen at good rates. For the first time there were significant differences in pollen collection between plots with different moisture levels - pollen collection being higher in those at the low moisture level. On the other hand, bee populations were higher on the high moisture plots. Yields were slightly better on the high moisture plots, indicating that the higher bee populations made up for the lower percentage of pollen collectors. In the seasonal trend the curve for tripped flowers followed the curve for bee population better than it did for the percent of pollen collectors. Apparently, it is possible to alter cultural practices to increase pollen collection, but the problem is to accomplish this without sacrificing seed-setting vigor of the plants and attractiveness to bees in general.

Pollen trapped from bees in the Howell nursery for several years showed that first-crop alfalfa bloom encountered much less competition from other pollen sources than second crop, in spite of the greater number of kinds of competing pollens on the first crop. Although the percentage of alfalfa pollen collected was greatest in the year when competition from other pollens was least, there was less total pollen

collected in 1961 than in some other years. Examination of the data for 1961 by 5-day periods shows a positive correlation between competing pollens and alfalfa pollen collected. This would suggest that either the presence of large amounts of pollen in the field stimulated pollen collection in general or, more likely, that colony strength and pollen-collecting weather were better during the period when pollen collecting was high. In any case, there was nothing to indicate any negative effect of "competing" pollens on the amount of alfalfa pollen collected.

At Logan, Utah, 20 colonies were divided into five groups of four each and fed the following materials: (1) Extract of alfalfa pollen evaporated onto sucrose crystals, (2) water extract of alfalfa pollen, (3) water extract of mixed pollens, (4) water extract of alfalfa flowers, and (5) sugar syrup. The colonies were placed in an alfalfa field at a time when the percentage of bees collecting alfalfa pollen was remarkably high (8%).

During the treatment period the colonies receiving sugar syrup brought in slightly less total pollen than the others and during the post-treatment period, slightly less total pollen. However, differences were so small that not much importance can be attached to them at this time. As usual in experiments with colonies next to alfalfa, some consistently gather more alfalfa pollen than others. This points to the need for a larger number of colonies than were used in this experiment.

A four-year experiment at Logan to determine the degree of isolation required to prevent cross-pollination between varieties of alfalfa was concluded in 1961. The gap between the blue- and white-flowered fields was increased to 15 rods. In spite of this the percentage of "contaminated" seed in the white field was higher than in the preceding years when the gap was smaller. This resulted from crossing with blue-flowered plants that volunteered in the white-flowered field. The spring provided ideal conditions for germination and, apparently, most of the cross-pollinated seed, which had shattered out and accumulated during the previous 3 years, germinated. The contaminated seed harvested in 1961 was progressively less as the distance from the blue-flowered plot increased. This indicates a cumulative effect of 4 years in which the same thing occurred. The experiment in its final year indicated that isolation distances greater than those used in the test are necessary for seed purity, and that there is the danger of leaving a seed-producing stand in production long enough for offvariety seed to accumulate and volunteer.

At Logan, into one cage placed over white-flowered alfalfa was introduced a honey bee colony which had spent a week where there was no alfalfa. Another similarly-placed cage received a colony taken

directly from a blue-flowered alfalfa field. A third cage had a colony which could forage both inside the cage and outside where blue-flowered alfalfa was growing. The percentages of contamination of the white with the blue flowered alfalfa in the three cages in the order given were as follows: 0.356, 1.79, and 1.17. The first figure was surprisingly high since no contamination was expected. The second appeared reasonable. The third was surprisingly low since a high percentage of contamination was anticipated. Perhaps the explanation for the low percentage in the third treatment was that the bees that worked outside the cage did not work inside and vice versa. The contamination in the first cage could not be explained.

In other work at Logan, bees were marked and followed in three different areas, (I) in the middle of a large field of alfalfa, (2) at the edge of the same field, and (3) on a small patch of alfalfa in an area with other small patches. The number of marked bees that was found and followed for several visits showed that in the order listed above the bees had progressively less difficulty returning to the same spot in the field.

In cage tests at Laramie, Wyo., in cooperation with the University of Wyoming, counts of honey bees foraging on square-yard areas usually showed 2 to 7 times as many bees on alfalfa blossoms inside screen cages as in the open field. Only 1.07% of the honey bees inside, and 0.66% outside, were collecting pollen, but 94% had pollen in their proboscis fossae, showing that nectar-collectors also distribute alfalfa pollen. Seed yields of the 7 commercial varieties of alfalfa and 7 experimental crosses under test usually were 3 to 9 times greater per row inside the cages with a colony of honey bees confined throughout the blooming period, as in the open field within flight range of a commercial apiary. Negligible seed was set inside a cage excluding insect pollinators. The percentage germination of a given variety was similar whether the seed was set inside or outside the cages.

In a study of alfalfa seed production at high altitude (7200 feet), also in cooperation with the University of Wyoming, it was shown that honey bees can definitely pollinate alfalfa at this altitude. Seed yields inside a screen cage to which a colony had access were more than twice as great per 100 stems, as yields from the open field within flight range of the same colony and a small apiary. Negligible seed was set inside a cage excluding insect pollinators.

The following manuscripts reporting results of research on honey bee pollination have been written and are in press: "Seed Crops Pollination by Insects," G. E. Bohart and Frank E. Todd, World Seed Campaign News; "Interactions among Foraging Honey Bees from Different

Apiaries in the Same Field," M. D. Levin, Insectes Sociaux;
"Pollination and Pollinating Agents of the Saguaro," S. E. McGregor,
Stanley M. Alcorn, and George Olin, Ecology; and "Pollination
Requirements of the Organpipe Cactus," Stanley M. Alcorn,
S. E. McGregor and George Olin, Cactus and Succulent Journal.

5. Pollen Analysis. At Tucson, Ariz., the lipids of dandelion, Taraxacum vulgare, and cottonwood, Populus fremonti, pollens were solvent extracted and analyzed by column and gas-liquid chromatography. Total lipid content of dandelion and cottonwood pollens was 18.2% and 4.1% respectively. Fractionation of the unsaponifiable fractions by column chromatography showed that hydrocarbons made up 3.2% and 0.82%, and neutral and related sterols, 4.8% and 0.79% of total lipids, respectively, in dandelion and cottonwood pollens. Gas-liquid chromatographic analysis of methyl esters demonstrated the presence of II fatty acids in the saponifiable fraction of dandelion pollen. Caproic, capric, myristic, made up 1.7%; caprylic, oleic, 7.4%; stearic, behenic, 17.9%; lauric linoleic, 27.4%; and palmitic and linoleic, 45.5% of the pollen fat sample analyzed.

D. Wild Bee Pollinators

1. <u>Wild Bees</u>. At Logan, Utah, females of <u>Anthidium emarginatum</u>, <u>A. banningense</u>, <u>A. rodecki</u>, <u>A. utahense</u>, <u>A. edwardsii</u>, and <u>A. maculosum</u> were brought into a large greenhouse section to see if they would nest in such an environment. Concurrently, artificial nesting sites were established in the greenhouse and in the field. These sites consisted of small holes in wood, soil, and clay blocks. In most cases the holes in the materials were fitted with straws.

In the greenhouse <u>banningense</u> and <u>emarginatum</u> nested in vertical holes in soil and clay blocks but rejected holes in wood and all horizontal holes. The other species apparently did not nest. In the field artificial nests were also accepted by <u>utahense</u> and <u>mormonum</u>. In addition, natural nests of <u>edwardsii</u> were found.

Observations were made on collection and use of pollen, nesting materials, nest arrangements, cocoons, mating activities, sleeping, and parasites. One undetermined species of <u>Stelis</u>, an anthidiine parasitic bee, was reared from an <u>Anthidium</u> nest in the field. This was the only parasite taken.

The following manuscripts reporting results of this research are in press: "Observations of the Visits of Honey Bees and Bumble Bees to Bladder Senna (<u>Colutea arborescens</u> L.)," W. P. Nye and G. E. Bohart, Annals Ent. Soc. Amer., and "Introductions of Foreign Pollinators — Prospects and Problems," G. E. Bohart, Proc. Intern. Sym. Pollination.

E. Effect of Pesticides on Honey Bees

1. Insecticide Studies. At Logan, Utah, a new dead-bee trap was designed which had a 99% efficiency. Insecticides were tagged with several types of dyes. Shilling's yellow food color was most satisfactory. Six observation hives equipped with removable hardware cloth sides allowed access to the bees. The weather during the tests (September I - 15) was windy, cool, and overcast, very unusual for the area. Trained bees were fed I ml. phosphamidon syrup at (I) .479 mg./ml., (2) 4.79 mg./ml., and (3) .0479 mg./ml. The lowest level was nontoxic and the intermediate was toxic only when fresh. Only one bee was fed the highest level and she was driven into the trap by the other bees while she was still alive.

The following attributes of nectar-producing plants in Utah were favorable for studies of systemic insecticides: (I) Daily production of 10 or more good-sized flowers per plant, (2) an accessible and visible amount of nectar of intermediate sugar concentration, (3) adaptation to greenhouse conditions and culture, (4) short prebloom and long blooming periods, and (5) moderate size. Four plant species were tested at Logan. Of these Borago officinalis was the most satisfactory. Phacelia companularia was good except for blackening the hands and causing a dermatitis.

At Logan, insecticides were applied (I) as a spray on the plants to the point of runoff and (2) as granules to the soil around potted plants. Treatments included several dilutions of demeton, dimethoate, phorate, and phosphamidon. Dimethoate and phosphamidon were drawn into fine capillary pipettes, transferred to .2 ml. graduated serological pipettes, and then tested for their oral toxicity to honey bees. Treated bees were held in one—ounce souffle cups with moistened sugar cubes for food.

The order of toxicity of the three materials tested was dimethoate — phosphamidon — phorate. Demeton at .1% was nontoxic. Peak toxicity of dimethoate in rape nectar occurred 48 hours after treatment and toxicity lasted for at least 6 days. In <u>Phacelia</u> and <u>Borago</u> peak toxicity appeared in 24 hours and did not last as long. At 0.8%, phosphamidon toxicity in nectar was about the same as that of dimethoate at 0.1%.

Two field applications were made of dimethoate at I pound per acre. In both tests half the field was sprayed with plain dimethoate and half dimethoate plus Vatsol, an aqueous wetting agent. Following evening applications there was a measurable mortality to foraging bees for several days, but it diminished rapidly after the first day. It apparently lasted 3 days among bees visiting the field and 4 days among bees confined to a large pollination cage.

In connection with research at Beltsville, Md., an investigation was made of honey bee damage resulting from gypsy moth spraying in Vermont during which it was evident that some losses were caused by the spraying of fruit bloom and the dandelion cover crop on which the bees were foraging at the time of spraying. The insecticide used was Sevin and application was made at the rate of I pound of Sevin per acre in $l\frac{1}{2}$ gallons of No. I fuel oil. The spray was applied by airplane.

A range from heavy to negligible loss of field bees was found in the apiaries visited. In no instance was there a complete loss of colonies. Some loss of honey crop may have resulted due to the fact that field bees were lost just prior to the main clover bloom which produces a good nectar flow in this area.

2. Assays. In studies at Madison, Wis., samples of bees collected from every apiary visited in Vermont were tested by two methods of bioassay — one by application of extracts to honey bees, one by application of extracts to brine shrimp. These tests were made II to I3 days after the final spraying. This unavoidable extended period of time between final spraying and analyses reduced the possibility of demonstrating the presence of any residual Sevin insecticide. However, toxic material was demonstrated in bees from one apiary. Pollen analysis revealed the predominant pollens present in and on the bees to be apple, dandelion, and some clover. Disease analysis showed that at least seven samples were infected with Nosema disease, with one sample showing an extremely heavy infection.

In studies at Tucson, Ariz., chemical and bioassay tests of nectar samples from Dibrom, parathion, and parathion-Kelthane and DDT treatments were all negative. The case history of "insecticide dust drift" into an apiary was obtained with a 640-acre watermelon field was dusted with parathion 5%, Kelthane 2%, and DDT 3%. Bees one-quarter mile and more away were seriously damaged.

In cooperation with the Pesticide Chemicals Research Branch, Plant Products Laboratory of the Eastern Utilization Research and Development Division, and the Abbott and Pfizer laboratories, the Madison, Wis., station conducted investigations on the possibilities of residues occurring in honey from chemicals and drugs used by and having value to the beekeeping industry. Except for phenol, contaminated surplus honey does not result when any of the essential chemicals and drugs are used at the proper time at the required dosage. Sulfathiazole, streptomycin, and fumagillin are quite stable in honey. These could cause contamination if employed close to or during the honey flow. However, they must be used I to 2 months in advance of the flow to have value in colony management. No satisfactory method has yet been developed to analyze honey samples exposed to the common

fumigants Methyl bromide, ethylene dibromide, paradichlorobenzene, and cyanide. Analyses for cyanide in honey proved exceedingly difficult but honey given the most severe exposure showed detectable levels of O.I p.p.m.

At Tucson, Ariz., nectar stored by small colonies placed within 16-acre alfalfa plots sprayed with one-fourth pound of parathion and one pound of Dibrom did not show detectable levels of the insecticides.

3. Protection of Honey Bees from Insecticides. In tests at Tucson conducted to study the value of covering colonies during spray application, parathion spray had a longer residual action than Dibrom. Covering of the hives for 1 or 2 hours after an application of parathion reduced the death rate to about a third that of uncovered colonies. This bee mortality was still considered far too high. More favorable results were obtained with Dibrom where the covered colonies had only a slight mortality rise.

Early morning applications of 6 highly toxic materials (Sevin, Zectran, dimethoate, phosphamidon, parathion, and Dibrom) were tried for comparison with mid-day and evening applications in previous experiments. While the morning treatments proved to be less severe on the colonies than those applied at other times of day, they were still too high for practical use around bees.

When colonies were exposed at dawn to aerial spray of Phosdrin no nectar could be shaken from the brood combs in the afternoon. Other colonies were covered with polyethylene when the spray was applied. Nectar shook freely from brood combs of these colonies.

At Tucson colonies were covered with burlap to simulate confinement for protection from insecticides in order to determine what effect such covering would have on the bees. Both wet and dry burlap of single and double thickness were used. Colonies were confined for as long as 36 hours when maximum outside temperatures exceeded 100° F. Colonies under the moist burlap remained quiet and their broodnest temperatures remained lower than those of control colonies.

Bees under dry burlap were less content, clustered outside the entrances, flew against the covering, and had higher broodnest temperatures than the control colonies had. There were about 200 dead bees at the entrances of the colonies under the wet burlap at the end of the tests and 230 to 300 at those of the colonies under the dry burlap.

Bees did not collect sufficient water to meet colony requirements from feeders placed at the entrance to the hive where it could be collected without flight.

4. Repellents. Four compounds highly repellent to honey bees in screening tests were applied to flowering alfalfa in Arizona. Reductions in the numbers of honey bee visitors were observed in all treated plots but in only one instance were the differences significant. Plant injury may have been of more importance than chemical repellency characteristics in reducing visitation.

At Logan, Utah, an attempt was made to use Phillips Company repellent R-874 to repel bees from certain plots in an alfalfa field. The bees were not repelled by this material. The good honey flow being experienced at the time may explain the fact that the bees remained on the bloom in spite of the repellent.

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AREA 19. ANALYSIS, SYNTHESIS, FORMULATION AND EVALUATION OF INSECT CONTROL CHEMICALS

Problem. Modern insecticides are at present the most rapid and effective means of controlling injurious insects and their use has enabled the American farmer to produce an abundance of high quality crops and livestock. This extensive use, however, has been accompanied by increasing resistance of some insects to certain insecticides and by the possibility of leaving harmful residues on or in harvested crops, in meat, or in dairy and poultry products unless the materials are used in accordance with recommendations. There is therefore a need for the development of new types of chemicals, from natural sources and through synthesis, to which insects will not become resistant. These chemicals should be safe to handle and not leave harmful residues in the harvested products used for foods or feeds, or adversely affect wildlife, beneficial insects and other desirable organisms. More effective formulations of chemicals should be developed for the control of different insect species under various environmental conditions. Such chemicals and formulations require initial testing in the laboratory and evaluation under field conditions before they can be recommended for practical use. It is necessary that accurate, sensitive analytical methods be developed for the determination of the amounts of chemicals deposited and the rate of disappearance of their residues and breakdown products in treated crops, animals, or soils. Better attractants are needed for use in traps and bait sprays for both insect detection and control. Research also is needed on repellents that would be useful in controlling insect attacks on crops, livestock, and man.

USDA PROGRAM

The Department has a long-term program involving chemists and entomologists engaged in both basic and applied research to discover and develop new and improved insect control chemicals and methods of applying them. Chemical research to discover, isolate, and identify products of natural origin which can be employed for insect control is carried on mainly at Beltsville, Md.; investigation of components of the cotton plant that serve as attractants or essential nutrients, or otherwise affect the boll weevil is in progress at State College, Miss., in cooperation with the Mississippi Experiment Station. Chemical research on synthetic organic materials and formulations for insect control is carried out at Beltsville, Md., Orlando, Fla., and State College, Miss. Development of analytical methods for insecticide residues is carried on at Beltsville, Md. (headquarters); Tifton, Ga.; Vincennes, Ind.; Moorestown, N. J.; Kerrville, Tex.; and Yakima, Wash. There is cooperation with the State Experiment Stations in the respective regions of all these laboratories. Cooperative work with the States on insecticide residues is carried on in connection with the following Regional Research Projects: NC-19. Fundamental Problems Associated with the Accumulation of Pesticidal Chemicals in Soils; NC-33. Pesticide Residues on or in Food, Feed, and Forage Crops; NE-36. Determination of Pesticide Residues on Raw Agricultural Commodities;

S-22. Pesticide Residues on Plant and Animal Products and Soils; and W-45. Pesticide Residues, Their Nature, Distribution, and Persistence in Plants, Animals and Soils. Research on aerosols for insect control is conducted at Beltsville, Md. A line project on soil fumigants at Moorestown, N. J. was brought to completion and terminated in 1962. Biological evaluation of insecticides and other types of insect control chemicals is carried on at Beltsville, Md., and Brownsville, Tex. Research on methods for control of insects in aircraft is done at Beltsville, Md.

The Federal scientific effort devoted to research in this area totals 39.2 professional man-years. Of this number 6.5 are devoted to products of natural origin as sources of insect control materials; 12.5 to development of synthetic organic materials and formulations for insect control; 7.0 to methods of analysis for insecticide residues; 2.2 to fumigants and aerosols for insect control; 8.0 to biological evaluation of insect control chemicals; 1.0 to methods for control of insects in aircraft; and 2.0 to program leadership.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 23.1 professional man-years divided among subheadings as follows: Products of natural origin as sources of insect control materials 1.4; development of synthetic organic materials and formulations for insect control 5.0; methods of analysis for insecticide residues 10.4; fumigants and aerosols for insect control 0.4; and biological evaluation of insecticides 5.9.

Industry and other organizations also conduct research in this area. A number of large insecticide companies carry on the synthesis and screening of compounds in a search for new and patentable insect control agents and develop various formulations of those compounds that show sufficient promise for commercial exploitation. They supply small samples of many of these compounds to Federal and State laboratories for evaluation. They also develop and utilize analytical methods to obtain data on residues necessary for Federal registration of their products and establishment of legal residue tolerances. Several research institutes and private research laboratories conduct work financed by industry on insecticides. Estimated annual expenditures of industry and other organizations are equivalent to approximately 400 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Products of Natural Origin as Sources of Insect Control Materials

1. Insect Sex Attractants. Encouraging progress has been made in the search for natural insect sex attractants that might be useful for survey or control of important insect pests. At Beltsville, Md., extracts have been prepared from male and female insects of a considerable number of species and made available for testing by cooperating entomologists. The presence of sex attractants has been demonstrated in adult, virgin females of the

tobacco hornworm, southern armyworm, salt marsh caterpillar, pink bollworm, and hessian fly. Research aimed at isolation and identification of these attractants is underway or planned.

The synthetic gypsy moth attractant, gyplure, developed by this project, which is a homolog of the recently identified natural gypsy moth sex attractant, has been successfully put into production by a commercial company. It was used in the 1961 and 1962 survey trapping programs of the Plant Pest Control Division in New England and compared well in effectiveness with the natural attractant. Recently gyplure has been separated into two geometric isomers; of these the <u>cis</u> form is an active attractant and the <u>trans</u> form is inactive.

- 2. <u>Insect Hormones</u>. Investigation at Beltsville, Md. of the insect juvenile hormone, which inhibits development to the adult stage, and the metamorphosis hormone (ecdysone) has continued. Large quantities of screwworm pupae and grasshoppers have been extracted to obtain ecdysone. A report that farnesol and farnesal, two acyclic terpenes isolated from feces of <u>Tenebrio</u> beetles, show juvenile hormone-like activity was confirmed and another related terpene, nerolidol, was also shown to be active.
- 3. Materials of Plant Origin for Insect Control. This work has been conducted at Beltsville, Md. The seed oil of the wild sesamum plant, Sesamum angolense, contains two compounds that synergize the insecticidal effect of the pyrethrins. One of the compounds, sesamolin, had previously been found in S. indicum seed oil. The other is a previously undescribed compound; it has been named sesangolin and has a structure closely related to both sesamin and sesamolin. Sesangolin is about equal to sesamin in its synergistic action with the pyrethrins.

A small amount of demissine, a crystalline alkaloid, was prepared from Solanum demissum leaves for evaluation as an insect chemosterilant. Demissine has been reported to be mutagenic to Drosophila.

An ether extract of <u>Sassafras albidum</u> leaves and stems was attractive to both male and female <u>Mexican fruit flies</u> in laboratory tests. The extract has been fractionated for further investigation.

Extracts have been prepared from several species of plants that have been reported to be attractive to insects. Among these were jimson weed, Datura stramonium, flowers, reported attractive to tobacco hornworm moths; Euonymus kiautschovica flowers, attractive to several species of flying insects; and beechwood creosote, attractive to mosquitoes.

- B. <u>Development of Synthetic Organic Materials and Formulations for Insect</u> Control
- 1. Preparation of Synthetic Organic Compounds for Testing as Insect Control Agents. Research at Beltsville, Md., and Orlando, Fla., on insect chemosterilants, chemicals capable of completely suppressing the reproductive

capacity of insects, has received increasing emphasis. A considerable number of compounds have been synthesized for evaluation for this purpose. Since there appears to be a definite relationship between antitumor activity and insect sterilizing properties, cooperative arrangements for the exchange of compounds have been established with the Cancer Institute of the National Institutes of Health and with a number of university laboratories that supply compounds for the NIH Cancer chemotherapy screening program. In addition several chemical and pharmaceutical manufacturers are supplying samples of experimental compounds for screening as insect chemosterilants. Compounds of several classes, notably aziridine derivatives, have been highly effective under laboratory conditions and in limited field tests. Many of the aziridine compounds polymerize rather easily, with a loss of chemosterilant activity, and methods therefore have been developed for their assay. Ways of purifying them and determining their stability with changes in pH are being studied.

Research to develop improved attractants for the detection, survey, or control of insect infestations has continued. It had previously been found that some amino acids were somewhat attractive to fruit flies. L-Lysine in the free base form is one of the most attractive, its attractancy for three fruit fly species being roughly proportional to concentration.

Several new amides that were prepared showed promise as insect repellents.

A large number of compounds were synthesized or obtained from cooperating agencies or companies and submitted to cooperating entomologists for screening of their insecticidal or synergistic properties. In initial laboratory tests about 30 of these were highly toxic to body lice and about 25 were very effective in killing mosquito larvae.

An investigation of the metabolites of Cl4-labeled deet (an outstanding insect repellent developed by USDA) in the urine of guinea pigs was started.

Assistance was given, in regard to agricultural chemicals, to the preparation of a lexicon of nonsystematic and trade names of organic chemicals which is to be published by the Synthetic Organic Chemicals Manufacturers Association.

2. Research on Formulations. In the investigations directed toward development of improved formulations of materials for insect control, conducted at Beltsville, Md., and Orlando, Fla., attention was given to further improvement of physical standards and testing methods for granulated insecticides. A turntable device was developed for more accurate and representative sampling of granulated formulations and was later modified for sampling large lots. Its use was incorporated in purchase specifications for granulated insecticides. Cooperation was given to the National Agricultural Chemicals Association in their recently initiated study on problems of standardization of granulated pesticides. A number of experimental granulated formulations of attractants and insecticides were prepared for testing by cooperating entomologists. A revised specification prepared for granulated heptachlor was issued by the General Services Administration as

Interim Federal Specification 0-I-00528a.

The evaluation of respirators and gas mask canisters for protection against dusts, mists, and low vapor concentrations of pesticides has been kept up to date by testing the available devices against new pesticides that are proposed for use. This information is needed by the Pesticides Regulation Division in connection with the registration of new materials. Many Federal and State agricultural and health agencies and other organizations concerned with pesticide use also request this information. A report containing the latest data on this subject is in the process of publication.

C. Methods of Analysis for Insecticide Residues

Two analytical methods were developed for the determination of dimethoate residues. One is based on oxidation of the residue to a sulfone, alkaline hydrolysis of the latter to yield formaldehyde, and measurement of the color produced when the formaldehyde reacts with chromotropic acid solution. The other is based on treatment of the residue with alcoholic alkali, followed by development of a color with 1-chloro-2,4-dinitrobenzene.

A sensitive colorimetric method has been developed for determination of endosulfan (Thiodan) residues. This is based on reaction of endosulfan with alkali to form a compound that gives a quantitatively measurable color with aqueous pyridine-alkali solution.

A colorimetric method for phorate residues previously developed in this project has been improved to increase its sensitivity.

A method for determining residues of the acaricide Hooker HRS-16 (Pentac) has been worked out, involving combustion and automatic coulometric titration of the chloride ion.

At the request of the Federal Housing Administration, a rapid, simple method has been developed for determining aldrin and chlordane in soils treated for termite control. This method is being adopted by the FHA for use by its inspectors to ascertain whether treatment specifications have been met.

In cooperation with the Poultry Research Branch of the Animal Husbandry Research Division, considerable effort has been devoted to the development of analytical methods for terephthalic acid residues in chicken tissues. This compound is used as a synergist for certain antibiotics in the treatment of chronic respiratory disease, which is a serious problem in the poultry industry. Work is continuing on the evaluation and refinement of these methods.

An emergency investigation has been initiated at the request of the Pesticides Regulation Division, on the conditions under which trichlorocarbanilide (TCC), a bacteriostat used in some detergents, is hydrolyzed to chloroanilines. Several cases of cyanosis ("blue babies") have been reported in premature infants wearing diapers that had been washed with TCC-

containing products and autoclaved. The chloroanilines are known to produce cyanosis. Methods of analysis have been established and it has been shown that some mono- and di-chloroanilines are formed when detergent solutions containing TCC are boiled. It remains to be determined whether any chloroanilines are left in clothes washed with these detergents under practical conditions.

Investigations of residues in crops, animal products, and soils employing the analytical methods developed are described under other appropriate areas of Entomology Research Division.

D. Fumigants and Aerosols for Insect Control

1. Aerosol Formulations. Problems involved in the formulation and storage of aerosols containing DDVP, an organic phosphate insecticide of low mammalian toxicity, and related compounds have been studied at Beltsville, Md. The selection of the container, propellent, and solvent to be used is important, because these phosphates react with many of the common halogenated solvents, alcohols, and ketones and attack metal containers. The moisture content of DDVP aerosol formulations is critical in the reaction with the metal. Below 30 p.p.m. of water there was no deterioration even after 7 months storage at room temperature or at 110° F.

A small aerosol container with a break-off tip nozzle was satisfactory for use in disinsection of aircraft. A number of these containers were made and filled with formulations to be used in cooperative tests with the World Health Organization on aircraft disinsection procedures.

Pyrethrum-DDT aerosol formulations containing different solvents were prepared for tests of their comparative irritating properties for aircraft passengers. Highly concentrated pyrethrum extracts, containing up to about 80% of pyrethrins, were compared with the usual 20% extract and showed no difference in irritating qualities. In investigating aerosol formulations with increased pyrethrin content it was found that the content could be doubled without changing the particle size of the aerosols by using an extract containing 40% pyrethrins.

There has recently been much interest in the aerosol industry in developing water-emulsion formulations using low-boiling hydrocarbons as propellents for pressurized sprays. A study is being made of this type of formulation with respect to stability and safety, and the effects on valve components and containers.

In cooperation with the Pesticides Regulation Division of ARS, the Aerosol Division of the Chemical Specialties Manufacturers Association, and the New York City Fire Department a study is in progress to evaluate and recommend test methods for determining the flammability of aerosols. A reliable and sensitive method is particularly needed for new types of aerosol formulations containing volatile hydrocarbons as constituents.

A number of experimental aerosol formulations of new insecticides have been prepared for tests against greenhouse pests, flies, mosquitoes, and other insects.

- 2. <u>Fumigant Formulations</u>. Another line of research has been the development of fumigant formulations for use against immature stages of soilinhabiting insects. Emulsifiable formulations of several new insecticides were prepared for tests as soil treatments against Japanese beetles. This work was discontinued in April 1962.
- E. Biological Evaluation of Chemicals for Insect Control
- 1. <u>Insecticides</u>. One of the major activities in this area is the initial laboratory testing of synthetic organic compounds and natural products against several species of insects to determine whether the materials have insecticidal, synergistic, attractant, repellent, insect chemosterilant, growth controlling, or other effects that would be useful for insect control. This testing is followed by a distribution of materials that have shown evidence of effectiveness to 40 field laboratories of the Entomology Research Division for more extensive secondary evaluation against about 125 species of insects and mites. Some of these materials are prepared within the Pesticide Chemicals Research Branch and others are supplied by other government or private research agencies or by industry.

Nearly 900 compounds were subjected to preliminary screening tests as insecticides during the year. The more effective of these were then distributed for secondary testing. Thirty-two of them showed high toxicity to at least one species of insect or mite.

A laboratory culture of the face fly, <u>Musca autumnalis</u>, which was successfully established for the first time during 1960, has continued to grow well and reached numbers sufficient for some testing of materials for the control of this pest. A number of compounds have been tested in the laboratory. A new culture of these flies also was started during 1961 from field-collected flies and has been successfully maintained. Field tests were made against face flies on dairy cattle with several experimental pressurized spray, dust, and bait spray formulations. The best results were obtained with a Dibrom bait spray, but its use cannot yet be recommended because this compound has not been cleared and registered for use on dairy animals.

2. Materials That Control the Activities of Insects Through Effects Other Than Death. Increased attention has been given to the evaluation of synthetic and natural materials for properties that could be used to control insects through effects other than kill. In cooperation with the chemists a search was initiated at Beltsville, Md., into sources of the so-called juvenile hormone which inhibits the maturation of insects. In connection with this a program of rearing insects common to the area which are useful for this purpose was undertaken and lepidopterous insects of the following genera are being reared: Cecropia, Polyphemus, Anisota, Protoparce, Datana,

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and Actias. Tenebrio molitor is being used as a test insect for evaluation of juvenile hormone effects. With this method of assay, juvenile hormone-like activity has been found for a crude extract of Cecropia and the compounds farnesol, farnesal, and nerolidol. A procedure for the bioassay of ecdysone, an insect metamorphosis hormone, has been adopted which uses Calliphora larvae as test organisms. A convenient and efficient method of bioassay has been developed for the American cockroach sex attractant.

Research carried out at Brownsville, Tex., has demonstrated the production of a sex attractant by the female southern armyworm moth. A procedure has been developed for extracting the attractant and cooperating chemists have prepared various fractions from the extracts for further study. A sex attractant also has been extracted from the pink bollworm. In connection with the sex attractant studies, features have been determined for the recognition of male and female insects in the pupal stage. Drawings showing the distinguishing features have been prepared for the southern armyworm, the bollworm, the tobacco budworm, the pink bollworm, the salt marsh caterpillar, the cotton leafworm, and the cabbage looper. At Beltsville, Md., the presence of a sex attractant was demonstrated in the female bagworm, Thyridopteryx ephemeraeformis.

3. Aerosols and Space Sprays. Tests conducted with sprays and aerosols containing DDT in combination with N,N-dibutyl-p-chlorobenzenesulfonamide, a compound developed by the Wisconsin Alumni Research Foundation under the name WARF antiresistant, showed a high degree of synergism against DDT-resistant house flies. Comparative tests of the aerosol formulation adopted by the World Health Organization for aircraft disinsection (0.4% pyrethrins and 3% DDT) and the G-l152 aerosol used in the United States for that purpose (1.0% pyrethrins and 3% DDT) showed that the two gave equal kills of nonresistant house flies and that both were much less effective against resistant house flies. An aerosol containing 0.4% DDVP, which was received from the World Health Organization for tests, was less effective than the Official Test Aerosol (0.4% pyrethrins and 2% DDT) against resistant and nonresistant house flies.

Tests were initiated with some of the new water-emulsion type aerosol formulations propelled by low-boiling hydrocarbon gases which are being introduced by the aerosol industry. One formulation recently accepted by USDA for registration contained allethrin, DDT, methoxychlor, Thanite, and MGK-264 and was propelled by isobutane, propane, and propellent 11. It equaled the Official Test Aerosol against nonresistant house flies but was ineffective against resistant flies.

In tests against the blow flies, <u>Calliphora vicina</u> and <u>Phaenicia sericata</u>, the Official Test Aerosol was highly effective. The Federal Specification aerosol and the G-l152 aircraft aerosol also were effective against \underline{C} . <u>vicina</u>.

F. Methods for Control of Insects in Aircraft

In cooperation with the Plant Pest Control Division, tests were made with a DDT dust applied in aircraft with a special applicator that had been developed at Moorestown, N. J. by research workers in the Entomology Research and Plant Pest Control Divisions. The object of this treatment was to prevent the spread of Japanese beetles by aircraft travel from the east to the west coast of the United States. Satisfactory results were obtained from the treatment and it was adopted for use in connection with the Japanese beetle quarantine.

Small one-shot aerosol dispensers with break-off tips have been proposed for aircraft disinsection and were tested under actual operational conditions. Formulations containing a mixture of pyrethrins and allethrin were dispensed from these containers in Military Air Transport 707 jet aircraft. Complete mortality was obtained of <u>Aedes aegypti</u> in cages located throughout the aircraft when 100 mg. of pyrethrins plus 100 mg. of allethrin made up to 10 cc. with propellent 12 was applied from 6 one-shot dispensers located 15 feet apart in the aircraft or one dispenser per 1,333 cubic feet.

Cooperative tests were carried out with the World Health Organization on the aerosol treatment of aircraft at "blocks away" (just after the door is closed for departure). The one-shot, break-off tip aerosol dispensers mentioned above were used and two formulations were compared, the WHO standard reference aerosol (SRA) containing pyrethrins and DDT with xylene and odorless petroleum distillate as solvents, and G-1480 containing pyrethrins and DDT with Velsicol AR-50 and AR-60 as solvents. The dosage of G-1480 applied was adjusted to give three times the pyrethrins and twothirds the DDT obtained with the recommended dosage of the SRA. The treatments were made by crew members on regular airline flights from London to Baltimore, London to Geneva, and Rome to Geneva. Caged mosquitoes of the following strains placed at locations throughout the planes, were used as test insects: nonresistant Aedes aegypti and Culex fatigans, DDT-resistant A. aegypti and C. pipiens, nonresistant Anopheles stephensi and A. gambiae. The SRA gave satisfactory control of the nonresistant mosquitoes. G-1480 was effective against both the resistant and nonresistant mosquitoes, but was irritating to some of the passengers. Work is now in progress on an improved formula effective against the resistant insects and nonirritating to passengers which will be tested during the summer of 1962.

A capacity-sensing device has been used with a recorder to study the diurnal rhythm of the locomotor activity of the cockroach <u>Leucophaea</u> <u>maderae</u>, without any electrical attachments to the insect. Movements were recorded over a period of weeks under natural conditions of daylight and dark, continuous complete darkness, and continuous light. Such monitoring apparatus might be useful for studying the activity of insects or other biological organisms in space flights.

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AREA 20. IDENTIFICATION OF INSECTS AND RELATED ARTHROPODS

Problem. Only about a third of the estimated two million or more kinds of insects in the world have been described and named. Many of these are of no immediate concern to agriculture or mankind, but thousands of species are potentially destructive or useful. Minute morphological differences are very important in recognizing many species, and only highly trained specialists are able to positively identify known species and describe new ones. Precise information on the identity and distribution of insects is essential to the efficient conduct of programs concerned with research on harmful insects and the development of methods for their control, and in the management of regulatory activities intended to exclude, control, or eradicate insect pests.

Knowledge of the classification and identification of insects at present is far from adequate. Knowledge of the insect fauna of the world provides the best assurance that any potential pests will be immediately recognized, so that appropriate safeguards can be set up to exclude them or prompt action taken to control or eradicate them if accidentally introduced. Moreover, with increasing emphasis on the utilization of beneficial insect parasites and predators to help control destructive insects, it is necessary that we know which insects to search for, where they might be found, and how to recognize those that may be useful.

USDA PROGRAM

This program of the Department is a long-continuing one involving insect taxonomists, and includes basic research to make known to science previously unrecognized and undescribed species of insects, ticks and mites, and the application of results of this research to the problem of insect identification. The work is carried on to a limited extent at Beltsville, Md., but mostly in Washington, D. C., in close cooperation with the U. S. National Museum of the Smithsonian Institution. Somewhat less active cooperation is maintained with various centers of taxonomic research in the United States and foreign countries and with numerous individuals in many parts of the world.

The Federal scientific effort devoted to research in this area totals 33.0 professional man-years. Of this number 7.5 is devoted to basic studies to name and describe beneficial and injurious insects, mites and ticks; 17.1 to identification of insects, mites, and ticks; 7.4 to preparation of keys and monographs on the classification, distribution, morphology, and biology of insects and related arthropods; and 1.0 to program leadership. Additional research in this area is provided by 1.5 professional man-years under P. L. 480 Project S9-ENT-6 (Uruguay) and 4 professional man-years under P. L. 480 Project F4-ENT-2 (Egypt).

A Research Contract with North Carolina State College of Agriculture and Engineering to catalogue a group of insects, Homoptera, Jassoidea, was terminated in July 1961 with the receipt of a manuscript covering the results of the research.

State Experiment Stations in 1961 reported a total of 22.9 professional man-years divided among subheadings as follows: Basic studies to name and describe beneficial and injurious insects, mites, and ticks 9.3; identification of insects, mites, and ticks 6.7; and preparation of keys and monographs on the classification, distribution, morphology and biology of insects, mites, and ticks 6.9. Most of the effort by Experiment Stations in this area is made in the North Central States - particularly Illinois - and in the Western States - particularly in California.

Industry and other organizations have a varying degree of interest in and contribute to the study of systematic entomology. Individuals who may be (1) members of staffs of universities, (2) employees of taxonomic units in State governments, (3) employees of museums which are not supported directly by the Federal Government, (4) recipients of research grants-in-aid, (5) individuals who earn their livelihoods at other work but study taxonomy as an avocation, and (6) retired persons, contribute to progress in this area. Estimated annual expenditures are equivalent to approximately 70 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

- A. <u>Basic Studies to Name and Describe Beneficial and Injurious</u>
 Insects, Mites, and Ticks
- 1. <u>Predaceous Mites</u>. Mites of the family Phytoseiidae are considered to be the most important predators of other groups of mites that feed on plants. It is probable that foreign species will be useful if introduced into the United States. A taxonomic study has been completed on the species of phytoseiid mites of Central Africa and is essential to an understanding of predatory species that occur in Africa.
- 2. <u>Cockroaches</u>. Two studies of cockroaches of Brazil made it necessary to describe as new 13 species among the 72 reported. Thirty-one genera, 2 of them previously unrecognized, were represented in the material. Many new locality records are included in the data with the specimens, and these records contribute important information to our knowledge of the geographical distribution of cockroaches.
- 3. <u>Plant-feeding Beetles</u>. Investigations were conducted in South America on plant-feeding beetles that are expected to be instrumental in suppressing alligator weed. Basic observations on habits, host-plant preferences, geographical distribution and structural differences, have developed essential understanding of the various species involved. An

important by-product of these observations is the development of a greater appreciation of the significance of field studies of living populations of insect species.

4. Entomological Glossary. Uniformity, or at least understandability, of terms used by entomologists throughout the world is necessary to progress in insect taxonomy. Available dictionaries seldom make important shades of meaning clear. Exact connotations can usually be gleaned only from the context of publications in which the terms are used. A glossary of entomological terms used by Polish, Czechoslovakian, Russian and other European entomologists, containing approximately 2,000 definitions, has been compiled and published.

B. Identification of Insects, Mites, and Ticks

Authoritative identifications and references to pertinent taxonomic and biological literature are supplied in support of Federal and State research, control and regulatory activities pertaining to entomological problems. These services are also performed for industry, pest control operators, and private individuals in the United States and for foreign agencies and institutions concerned with entomology.

During 1961 a total of 34,663 separate lots, mostly insect material, was received for identification. The staff examined well over 330,000 specimens and made and reported 94,674 identifications. Even though an attempt was made to keep up with the demands for identifications and material was not accepted unless there appeared to be a justification for the identifications, the backlog of material awaiting study and report continually increased.

The sources of material and the numbers of identifications made for each in the calendar year 1961 are shown in the following table:

	Number of	D
Source	<u>Identifications</u>	Percent of Total
Agricultural Research Service		
Plant Quarantine Division	20,558	21.71
Plant Pest Control Division	8,451	8.93
Entomology Research Division	8,939	9.44
Forest Service	2,438	2.58
Agricultural Marketing Service	5 28	.56
Other Federal Agencies	3,180	3.36
States and Insular Possessions	27,773	29.33
U. S. individuals	15,214	16.07
Foreign agencies and individuals	7,593	8.02
Total	94,674	100.00

Many specimens received for identification represented species not previously in the National Collection, or they documented new distributional data. For these reasons 73,415 specimens were added to the Collection during the year.

The systematic review of technical literature essential to the programs in this area included the examination of 1,617 publications which contained 3,382 articles of scientific interest. A total of 1,824 articles was catalogued. From these were prepared 6,476 index cards to authors and 18,356 cards on which were recorded actions of significance to taxonomists. Reasonably complete review of current literature is confined to the groups of insects having only the greatest economic importance.

During the year 122 visitors obtained aid on taxonomic or nomenclatural problems. The time that the visitors were present ranged from an hour or two to several months.

- C. <u>Preparation of Keys and Monographs on the Classification</u>, <u>Distribution</u>, <u>Morphology and Biology of Insects and Related Arthropods</u>
- 1. <u>Insect Biology</u>. Long term research is necessary to produce data which will demonstrate the importance of differing habits of insects as criteria for distinguishing species. Such data are being gathered on various groups of solitary wasps. Type of nest and gallery in the nest, the kinds of food with which wasps stock their cells, and the seasons of the year in which the insects are active, are significant indicators of different biological entities. Wasps are excellent subjects for establishment of principles which will also apply in other groups of insects.
- 2. <u>Two-winged Flies</u>. New genera and species were described in important groups such as the fruit flies, biting midges and bot flies. A supplement was issued to the synoptic catalog of the mosquitoes of the world. An important contribution to the knowledge of the mosquitoes of Alaska includes information on the distribution and identification of the species in that State. This information will help the military services as well as individuals to solve mosquito problems in Alaska.
- 3. <u>Beetles</u>. Research on the taxonomy of beetles has resulted in better knowledge of the immature or larval forms, as well as of the relationships of many species based on adult characters. Important information was discovered on the significance of anatomical differences and similarities displayed by some of the New World species of Tenebrionidae, a very large group of beetles. The weevil genus <u>Smicronyx</u> had not been studied as a whole since 1894. It was necessary to completely revise the group in order to make identifications of the species and organization of the biological data possible. The genus now contains 70 species in North America. At least 12 of these species are now known to attack the serious weed, dodder.

- 4. <u>Leafhoppers</u>. The Research Contract with North Carolina State College of Agriculture and Engineering resulted in the production of a 10,000-page manuscript entitled: "Catalogue of the Homoptera, Jassoidea."
- 5. <u>Grasshoppers</u>. Research under P. L. 480 Project S9-ENT-6 (Uruguay), entitled "Systematic collection, identification and classification of the grasshoppers of Uruguay and neighboring territories of Southern Brazil, Southern Paraguay, and adjacent provinces of Argentina" began in January 1962. New localities have been recorded for four grasshoppers and research on the biology of several species has been carried on.

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AREA 21. FOREIGN EXPLORATION, INTRODUCTION AND EVALUATION OF BIOLOGICAL CONTROL AGENTS

Problem. Many of the most serious insect and weed pests in the United States have been accidentally introduced from foreign countries without the insect enemies that kept them under control in their native homes. Some of the harmful insects so introduced have been effectively controlled by later introduction of their parasites and predators. Foreign exploration for beneficial biological control agents of insects and their subsequent introduction, colonization, and evaluation in this country is now a well established practice in the control of introduced insect pests. The use of imported insects to control introduced noxious weeds, although a more recent practice, has shown much promise. The biological approach to the control of insect and weed pests has great potential. Therefore, further foreign exploration is needed and additional research is necessary on the biology, ecology, nutritional requirements and the most effective manner of utilizing natural control agents, if they are to be used to maximum advantage. There is growing concern by the public over the insecticide and other residue problems in foods and by conservationists over the potential hazards of insect control chemicals to fish and wildlife. More effective use of natural control agents in meeting destructive insect and noxious weed problems could materially contribute to the ultimate objective of overcoming the pesticide residue and other hazard problems associated with the use of chemicals for the control of insects and weeds.

USDA PROGRAM

The Department has a continuing program on the use of beneficial insects. Basic and applied research is conducted on insect parasites and predators of insect pests and on insects that attack weeds, including foreign explorations for beneficial species and their introduction, liberation and evaluation in this country. A laboratory is maintained at Nanterre (near Paris), France, for studies on the parasites and predators of agricultural pests that have accidentally been introduced from Europe into the United States. At a station in Rome, Italy, studies are in progress on insects attacking a number of weeds, including puncture vine, Scotch broom, Dalmatian toadflax, Mediterranean sage, and Russian knapweed; and a similar station is maintained at Rabat, Morocco, for the study of insects that attack the weed halogeton. Three exploratory trips have been made by an entomologist to South America to search for insects attacking alligator weed, an important pest of waterways in southern United States, and in June 1962, a station was established at Buenos Aires, Argentina, to conduct further research on this problem. In 1962, an entomologist spent about 6 weeks in Israel collecting parasites of the brown soft scale, a recent serious pest of citrus in Texas.

In the United States a receiving station and laboratory is maintained at Moorestown, N. J., where major emphasis is given to receiving, propagating and transshipping insect parasites to proper liberation points. A laboratory for receiving, studying, and liberating insects affecting range weeds is located at Berkeley, Calif. Studies regarding entomophagous insects are also conducted at Riverside, Calif., and Beltsville, Md. The work at Berkeley and Riverside is conducted in cooperation with the University of California and the California Experiment Stations.

The <u>Federal</u> scientific effort devoted to research in this area totals 16.8 professional man-years. Of this total 2.8 is devoted to <u>search</u> for and importation of foreign parasites and predators of insect pests; 3.8 to <u>search</u> for and importation of foreign insect enemies of weeds; 5.8 to <u>basic biology</u>, physiology, nutrition and evaluation; 3.4 to receipt, liberation, and establishment of foreign insect enemies of insect pests and weeds; and 1.0 to program leadership.

Grants have been made for ten P. L. 480 projects that are directly concerned with the study of insect parasites and predators and their possible importation into the United States to help control introduced pests or closely allied species. Two projects, one in India and one in Pakistan, are for a study of the parasites and predators of rice insects; two in the same two countries are for work on parasites and predators of corn borers; and one in India is for an investigation of the parasites and predators of sugarcane borers. Two projects, one in Pakistan and one in Poland, are for a study of the biological control of scales and aphids on fruit. Three projects—in India, Brazil, and Uruguay—are for a general survey of the parasites and predators of agricultural pests. Altogether these projects call for 44 professional man-years annually. Since most of the projects have recently been activated, no extensive reports of progress are yet available.

Grants have been executed for two P. L. 480 projects for studies of insects attacking weeds. One is for a general survey in Pakistan. The other is for a study of insects affecting Striga (witchweed) in India. Two reports covering a full year have been received from Pakistan. They represent three professional man-years. No reports have been received from the Striga project in India as yet. It calls for 2 professional man-years annually.

State Experiment Stations in 1961 reported a total of 9.8 professional man-years divided among subheadings as follows: Search for and importation of foreign parasites and predators of insect pests 2.1; search for and importation of foreign insect enemies of weeds 0.5; basic biology, physiology, nutrition, and evaluation 3.4; receipt, liberation, and establishment of foreign insect enemies of insect pests and weeds 3.8. All except 0.6 of a man-year are reported from the Western

Region where California conducts an extensive program in biological control investigations. Special arrangements have been made between California and the Federal Government allowing California to import beneficial insects under strict quarantine regulations. California sends explorers to foreign countries to search for beneficial species, propagates and liberates desirable ones in large numbers, and conducts considerable applied and basic research on the biology, physiology and evaluation of insect parasites and predators. Under similar arrangements Hawaii has a program on biological control including foreign exploration and the propagation and release of desirable species. Oregon reports a small amount of work in this area.

Industry and other organizations conduct very little research in biological control. Most of industry's contributions in this area are made in California and Hawaii. Estimated annual expenditures by industry are equivalent to approximately 1 professional man-year. In some states the Universities conduct a limited amount of research on insect parasites and predators aside from that done at State Experiment Stations. Almost no work is being done by those institutions on insects affecting weeds. Estimated annual expenditures by Universities are equivalent to approximately 2 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

- A. <u>Search for and Importation of Foreign Parasites and Predators of Insect Pests</u>.
- 1. Parasites and Predators. Twenty-nine species of parasites or predators of 10 different insect pests were collected in Europe by the European Parasite Laboratory in France for shipment to the United States; five different parasites of the brown soft scale were collected in Israel; two species of parasites of the pea aphid and an alfalfa weevil parasite were collected in California; and an apple mealy-bug parasite was collected in Nova Scotia. In addition 22 species of parasites or predators of four introduced pests were collected in India and Pakistan through P. L. 480 projects. All of this material was sent to the Moorestown, N. J., laboratory for screening, testing, and transshipment to liberation points throughout the United States.
- B. Search for and importation of Foreign Insect Enemies of Weeds.
- 1. Enemies of Weeds. Two species of moths and two of weevils were imported from Europe and released against three weed species. These augmented releases made in 1959 and 1960 of two moths, Leucoptera spartifoliella, the larvae of which feed on Scotch broom, and Tyria jacobea, the larvae of which feed on tansy ragwort. The weevils were released for the first time against the puncture vine in several Western States.

Exploratory work in South America for insect enemies of alligator weed resulted in the discovery of a very promising flea beetle, Agasicles n. sp., and two other beetles of minor importance.

- C. Basic Biology, Physiology, Nutrition and Evaluation.
- 1. Face Fly. In a study conducted by the European Parasite Laboratory at Nanterre, France, no native parasites or predators of this insect were found in western Europe. Since the face fly has recently become a serious pest of cattle in the United States, a special effort was made by the Paris laboratory to obtain needed information on its life history, ecology and abundance under European conditions. This information will be very useful in investigations on the pest and its control in this country.
- 2. European Corn Borer. Over the years many efforts have been made to introduce and establish Campoplex alkae in the United States as a parasite of the European corn borer. Each effort has been unsuccessful, and it has been generally concluded that the species required an alternate host not present in this country. During 1961 a species of Campoplex was reared from the European pine shoot moth in Germany which appears to be "alkae." Experiments are being conducted to determine whether this important parasite may parasitize the shoot moth in the spring before a second generation attacks the European corn borer.
- 3. <u>Clover Leaf Weevil</u>. The fungus <u>Empusa sphaerosperma</u> has been considered the major biotic factor controlling the clover leaf weevil in the United States. Surveys conducted from Moorestown, N. J., however, have shown that the imported parasite <u>Biolysia tristis</u> is extremely important. Parasitization by this species in some fields ranged from 80 to 90%.
- 4. Lygus spp. Studies regarding the biological control factors affecting Lygus spp., have been initiated at Riverside, Calif., with special emphasis on insect parasites.
- 5. <u>Halogeton</u>. Life history studies and intensive screening tests were conducted at Rabat, Morocco, on <u>Heterographis fulvobasella</u>, an insect enemy of <u>Halogeton sativus</u>. It was hoped that this species might be useful in the control of <u>Halogeton glomeratus</u> in this country, but oviposition on dwarfed "glomeratus" plants in the greenhouse was not encouraging.
- 6. <u>Scotch Broom</u>. Detailed studies on the seed weevil <u>Apion</u> <u>fuscirostre</u> are being conducted at the Rome, Italy, laboratory. This weevil is of potential value as a suppressant of Scotch broom.

- 7. <u>Klamath Weed</u>. The status of the Klamath weed in California remains as previously reported. The weed persists only where available moisture along roads or in drainage areas supports recovery or on certain north-facing slopes or shady areas. Intensive studies are being conducted to determine why the major suppressant, the beetle, <u>Chrysolina quadrigemina</u>, shows a preference for the weed on southfacing slopes.
- 8. Gorse. Studies of the life history and biology of the seed weevil Apion ulicis, which feeds on gorse, are being conducted in California. This imported weevil increased enormously at San Rafael, Calif., and brought about a sharp decline in the gorse infestation at that location. In Oregon, where gorse is extremely abundant in the coastal areas, the effect of the gorse weevil is favorable in some areas but in others the plants show little indication of damage or decline due to feeding by the weevil. The number of seed pods produced in that area is small and possibly weevil populations are so low that the adult feeding is unimportant.
- 9. <u>Miscellaneous Insects</u>. A compilation of records regarding beneficial insects imported into the United States and a review of literature regarding the distribution and habits of beneficial insects of potential value to this country are being made at Beltsville, Maryland.
- D. Receipt, Liberation and Establishment of Foreign Insect Enemies of Insect Pests and Weeds.
- 1. Alfalfa Weevil. The alfalfa weevil has spread very rapidly in Eastern United States since it was discovered in that part of the country in 1952, and an attempt is being made to establish weevil parasites in the newly infested area as quickly as possible. One important European species, Bathyplectes curculionis, which was established many years ago in the West where the weevil has long been a pest of alfalfa, was collected in large numbers in California. Almost 32,000 were liberated in 10 Eastern States. Recoveries have been excellent and general establishment is proceeding well.

Four additional European parasites of the alfalfa weevil have been liberated. Two of them, <u>Tetrastichus incertus</u> and <u>Microctonus aethiops</u>, are definitely established. <u>Tetrastichus</u> is a tiny gregarious chalcidoid which parasitizes alfalfa weevil larvae. It is readily reared in the laboratory and almost 9,000 adults have been produced and liberated in 6 Eastern States. It has been recovered in Pennsylvania and New Jersey. <u>M. aethiops</u> is a parasite of adult weevils. Only 508 adults of the parasite were released, but the species has become established in New Jersey. <u>Dibrachoides druso</u> is a parasite of weevil larvae and prepupae. About 6,000 were released in 4 Eastern States, but it has not yet been recovered. The egg predator, <u>Peridesmia discus</u>, has not been recovered following liberation of 190 adults.

- 2. European Pine Shoot Moth. This pest causes serious damage in pine plantations. In recent years it has been particularly troublesome in the Midwest, and it has just been discovered in the Pacific Northwest. It is parasitized by a number of insects in Europe, about 15 species of which were released in Ohio and Michigan in 1961 and 1962. The principal species and numbers released in Ohio and Michigan, respectively, were as follows: Orgilus obscurator, 1,613 and 624; Pristomerus vulnerator, 996 and 1,529; Temelucha interruptor, 2,326 and 1,027; Tetrastichus interruptor, 1,570 and 3,526; Campoplex spp., 136 and 334. Itoplectis examinator (103 individuals) was released in Ohio only, and Lypha dubia (64 individuals) in Michigan only. It is too early to report on any recoveries.
- 3. Brown Soft Scale. This insect has become a problem on citrus in Texas during the last five years, reportedly because spray drift from cottonfields has caused severe mortality of the natural enemies of the scale. A number of useful parasites of the scale occur in Israel. One of them, Coccophagus cowperi, was obtained in sufficient numbers for rearing purposes and small colonies have already been released in Texas.
- 4. Apple Mealybug. The parasite, Allotropa utilis, was collected in Nova Scotia. A total of 1,220 were released in Vermont and 525 in Oregon. The parasite has already been recovered at the Vermont release site.
- 5. <u>Pea Aphid</u>. Releases of several different parasites were made against this pest. <u>Aphidius smithi</u>, an important parasite from India, has become well established in California, and collections made there were sent to several States as follows: Utah, 2,900; Colorado, 3,100; Arizona, 1,040; Oregon, 600; and New Jersey, 400. Several small colonies of European <u>Aphidius</u> were also liberated. A colony of 150 <u>A. avenae</u> was liberated in New Jersey; 411 <u>A. ervi</u> were liberated in Idaho; and colonies of 650 <u>Aphidius</u> sp., were liberated in Oregon and 127 in Arizona. The native eastern species, <u>A. pisivorum</u>, was released in Oregon (198) and in Washington (100).
- 6. <u>Balsam Woolly Aphid</u>. The balsam woolly aphid is a serious pest of balsam fir in the Northeast, in the Pacific Northwest, and on Mt. Mitchell in North Carolina. At the request of the Forest Service, the European Parasite Laboratory at Nanterre, France, made special collections of <u>Laricobius erichsonii</u>, a derodontid predator of the aphid in Germany for release in the United States. A total of 25,308 was shipped to Moorestown, N. J., where they were screened and transshipped to Maine (2,400), New Hampshire (5,040), Vermont (2,400), Oregon (2,281) and North Carolina (13,187). In addition 148 <u>Leucopis</u> sp., 43 <u>Metasyrphus lunulatus</u> and 15 undetermined syrphid flies were collected and sent to North Carolina.

- 7. Miscellaneous Insects. Small lots of parasites were released against several pests. Two hundred ninety-four Tiphia femorata were released against the European chafer in New York, 96

 Liodontomerus perplexus were released against the alfalfa seed chalcid in Arizona, and 11 Opius rhagoleticus were released against the cherry fruit fly in California. All of these are European parasites. In addition 807 native Opius ferrugineous were collected in the eastern United States for release against the cherry fruit fly in California. This parasite is considered to be generally distributed in the United States, but there may be differences between the eastern and western populations.
- 8. Scotch Broom. The twig mining moth, Leucoptera spartifoliella, was imported from Europe and first released in California in June 1960. Three additional releases totaling 5,054 moths were made in 1961. The moth is now firmly established at all five release sites in California. In addition it is well established at Tacoma, Wash., near Fort Dick, Del Norte County, in northern California, and in East Oakland, Calif.
- 9. <u>Puncture Vine</u>. Two species of weevils from Europe have been successfully introduced and established on puncture vine. One species, <u>Microlarinus lareynii</u>, attacks puncture vine seeds. The other, <u>M. lypriformis</u>, attacks the stems. About 1,500 seed weevils were released. They were colonized in California, Washington, Colorado, Utah, Arizona, and Nevada. About 1,200 stem weevils were released in the same States with the exception of Colorado. Recoveries have been made indicating that both weevils have successfully overwintered in California, that the seed weevil overwintered in Nevada and that the stem weevil overwintered in Arizona.
- 10. <u>Tansy Ragwort</u>. The cinnabar moth, <u>Tyria jacobea</u>, which feeds on tansy ragwort, has been successfully introduced into Washington, Oregon, and California from Europe. Additional releases totaling 3,750 larvae and 170 adults were made during this period. In Oregon, where initial releases of moths were made in 1960, several hundred larvae were counted at one site in 1961 and about 50 at another. In Washington no larvae were found in 1961, although they were observed in 1960. In California from 300 to 400 larvae were found in each of the 1959 release areas.
- 11. P. L. 480 Projects. Two Forest Service Projects, A7-FS-7 India and A17-FS-5 Pakistan, are concerned with the study of the insect enemies of Adelges spp. on fir. In this connection a considerable number of predators have been received and transhipped to two Forest Service Experiment Stations for experimentation and release against the balsam woolly aphid. The following were transshipped to Portland, Oreg.: 73 Coccinella 4-punctata, 55 Exochomus lituratus, 75 Adalia tetraspilota, 100 Ballia dianae, 10 hemerobiids and 454 Chrysopa sp.

The first four are coccinellids. The following were transshipped to Asheville, N. C.: 219 <u>Harmonia breiti</u>, 89 <u>Adalia tetraspilota</u>, 226 <u>Ballia dianae</u>, 67 <u>Ballia eucharis</u>, 110 <u>Oenopia sauzeti</u>, 32 <u>Adonia variegata</u>, 65 <u>Calvia</u> sp. (all coccinellids), 23 <u>Chrysopa</u> sp., and 77 <u>Leucopis</u> sp.

Investigations of parasites, predators and pathogens of sugarcane borers in India are being conducted under P. L. 480 Project A7-ENT-1 for which a grant was executed on July 25, 1961. A small colony of 87 Goniozus indicus, a gregarious chalcidoid parasite of borer larvae, was received from this project in 1962 and transshipped to the Grain and Forage Insects Research Branch laboratory at Canal Point, Fla., for study and experimentation.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

<u>Search for and Importation of Foreign Parasites and Predators of Insect Pests</u>

None.

Search for and Importation of Foreign Insect Enemies of Weeds

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Receipt, Liberation and Establishment of Foreign Insect Enemies of Insect Pests and Weeds

Puttler, Benjamin. 1961. Introduction, colonization and establishment of <u>Bathyplectes curculionis</u> (Thom.) a parasite of the alfalfa weevil in the eastern United States. Journ. Econ. Ent. 54(5): 878-880.

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AREA 22. INSECT PATHOLOGY

Basic investigations on viruses, fungi, bacteria, nematodes and protozoa are needed to fully exploit the use of such microorganisms as an approach to insect control. There is much interest in the use of these natural insect-control agents to overcome the growing concern over chemical residues following the application of insecticides to agricultural crops and livestock, and the increasing resistance of some insects to certain insecticides. The utilization of pathogens to produce diseases in insect populations, and so reduce them and the damage they cause, is an approach that has already shown great promise. Microorganisms that are pathogenic for insects are generally very efficient when used properly. They are specific for their insect hosts and harmless to men and other vertebrates. Basic research is needed for a thorough understanding of insect pathogens, including their growth and nutritional requirements, their resistance to environmental factors, and their mutability and mode of action, both in the laboratory and the field. Such knowledge must be obtained before these organisms can be used effectively in the control of insect pests.

USDA PROGRAM

The Department's Pioneering Research Laboratory on Insect Pathology at Beltsville, Md., has a continuing basic research program on the growth, nutritional requirements and mode of action of viruses, bacteria and nematodes affecting insects. Studies are in progress on mutability-Induced changes in virulence of insect diseases, and resistance of insects to diseases, including studies of the effect of the environment on the pathogens. A comprehensive reprint library on insect pathology is being assembled. Collections of all sporeformers and viruses known to cause disease in insects are being obtained from worldwide contributors. A service involving the diagnosis of unhealthy insects is now available to all Division, State, and University laboratories.

The program includes collaborative studies with the Festicide Chemicals Research Branch on instrumentation for monitoring insect activity, internal temperatures of insects, and effect of gaseous atmosphere on metabolism and development of insects. Collaborative studies are also underway with the Pioneering Research Laboratory on Insect Physiology on the effect of microorganisms on insect sterole requirements, and with the U. S. Naval Missile Center on effects of cosmic radiation on pathogenicity and enzymology of insect pathogens and changes induced in their nucleoprotein makeup.

In addition, attempts to propagate <u>Rickettsiella popilliae</u> on mammalian tissue culture were carried out in cooperation with the Naval Medical Institute, National Naval Medical Center, Bethesda, Md. These studies should determine the effect of tissue culture passage on the virulence of the organism for its insect host. A study of the effects of a chemosterilant and gamma radiation on the reproductive tissue of <u>Drosophila melanogaster</u> is being carried out in cooperation with the Fruit and Vegetable Insects Research Branch. The Laboratory cooperates with insect pathologists at the Forest Insect Laboratory at New Haven, Conn., and the Forest Sciences Laboratory at Corvallis, Oreg. Contact is also maintained with insect pathology laboratories at Sault Ste. Marie, Ontario, Canada; Darmstadt, Germany; Versailles and Paris, France; and Prague, Czechoslovakia. Exchange of cultures and techniques are carried out routinely with these laboratories and informal cooperative research has been initiated with some of them.

The <u>Federal</u> scientific effort devoted to research in this area totals 9.0 professional man-years. Of this number 2.6 is devoted to <u>virus</u> <u>diseases of insects</u>; 4.5 to <u>bacterial</u>, <u>protozoan</u>, <u>and fungus diseases of insects</u>; 1.0 to <u>nematodes and their associated bacteria pathogenic to insects</u>; and 0.9 to <u>discovery and study of new pathogens</u>.

State Experiment Stations in 1961 reported a total of 8.6 professional man-years divided among subheadings as follows: Virus diseases of insects 3.7; bacterial and fungus diseases of insects 3.2; nematodes and their associated bacteria 0.7; and discovery and study of new pathogens 1.0. The State Experiment Stations are constantly adjusting their programs on the diseases affecting insect pests as new diseases are discovered within a State or new pathogens become available that might be useful. As new diseases are discovered, they are given careful scientific study.

Additional research (1.5 professional man-years) is in progress under a grant of P. L. 480 funds to the Institute of Plant Protection, Poznan, Poland, for the isolation and study of protozoan diseases of insects.

Industry. Several fermentation industries are producing bacteria (Bacillus thuringiensis) for control of insects. Extensive tests to improve production and increase yield are under way. Tests of pathogenicity for vertebrates are being conducted and other microorganisms are under study with the viewof ascertaining the optimal growth conditions and their pathogenicity for insects. Estimated annual expenditures are equivalent to approximately 30 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Virus Diseases of Insects

- l. Determination of Larval Instars. In order to determine extent of the prodromal phase of virus infection in insects it is necessary to have an accurate means of determining the several stages or instars of the larvae. Measurements of head capsules of larvae of the salt-marsh caterpillar, Estigmene acrea, were made to determine the number of instars in the larval developmental cycle. There are normally seven instars; however, stress, such as storage at low temperature, may induce an extra molt. The head capsule-sizing technique does not provide an accurate estimate of instars due to overlapping of measurements in the sixth, seventh, and eighth instars. This project has been terminated.
- 2. Time of Food Passage Through Insect Gut. It is important in histopathology studies of infected insects to estimate the location of ingested microorganisms in the gut at any given time after feeding. Investigations using two insects, the salt-marsh caterpillar, Estimene acrea, and the variegated cutworm, Peridroma saucia, were carried out using different colored foods. In the case of E. acrea, kale was fed for twenty-four hours followed by carrots. Kale and potatoes were used with P. saucia. Examination of the frass indicated the time of passage. The overall passage time did not indicate the length of time food remained in any one part of the gut. Investigations will be continued by feeding X-ray opaque barium sulfate with the food and X-raying the insects at regular intervals after feeding. These investigations will be extended to other insects and cooperative arrangements have been made to conduct X-ray tests on the gypsy moth, Porthetria dispar, with the Forest Insect Laboratory at New Haven, Conn.
- 3. Abnormal Growth in Virus-infected Tissue. Tumor formation in the gut of Gilpinia hercyniae infected with a nuclear polyhedrosis was described by F. T. Bird in Canada. No abnormal growth in healthy insects has ever been detected. It has been observed that during granulosis infection in the fat body of Choristoneura murinana mitosis occurs in uninfected cells. Investigations of healthy Estigmene acrea larvae immediately before the pupal molt has indicated that no mitosis of fat cells normally occurs at this time. A paper on the implications of these findings is in preparation. This work will be continued using granulosis-and polyhedrosis-infected E. acrea larvae to determine the extent of abnormal growth in relation to infected tissues. Such a characteristic or abnormal growth would be an aid in diagnosis of insect diseases and might lead to further work on the action of the virus.

4. Mode of Invasion of Virus in Insects. Most studies involving mode of action of viruses have been concentrated on the activity of the virus in the nucleus of the host cell where virus multiplication takes place. There has been practically no work done on the means by which the virus gains entrance to the cell. However, this phase of its invasion of the host must be of great importance with regard to virulence.

Insect virus particles are enclosed in a proteinaceous material (called polyhedral protein) which is remarkably resistant to the environment. Polyhedral protein is dissolved by alkaline, reducing conditions, and enzymes present in the insect gut, thus releasing the virus particles. Conditions in the insect gut very rapidly inactivate the naked virus, but little is known about the size and nature of the breakdown products. Experiments with devices to artifically introduce virus into the insect gut and retrieve the same preparations for chemical studies and electron microscopy are now underway. It is possible that the infective particle size may also be determined in this manner.

- 5. <u>Virus Culture Stock Collection</u>. A collection of all the reported viruses from insects throughout the world has been started. Whenever possible, the material will be tested against economically important insects on the North American Continent. These starting cultures are being used to build up significant quantities of the more virulent disease organisms.
- 6. <u>Electron Microscopy</u>. A Hitachi HU-ll electron microscope has recently been installed, and work on improved methods of imbedding, staining, and sectioning virus materials has been initiated.
- 7. Rickettsiella popilliae. Frozen suspensions of R. popilliae stored in dry ice for 9 years were infective at low dosages. Fresh bacteria-free suspensions of this rickettsia were prepared to augment a supply of this organism and to furnish Dr. Earl Suitor, U. S. Naval Medical Research Institute, with material suitable for chick entodernal explant, and human syrobial tissue culture studies. Dr. Suitor reported successful culture of the organism and arrangements are underway to test his cultures for retention of pathogenicity by injection of Japanese beetle larvae. Approximately 1,000 larvae were reared from eggs to provide a disease-free stock suitable for these tests. Dr. Suitor has also succeeded in culturing a similar organism from Melolontha melolontha. The cultures obtained from this stock will also be tested. Preliminary tests indicate that adults of the Japanese beetle are as susceptible as the larvae to blue disease infection and may be used as experimental animals in this study.

B. Bacterial Diseases of Insects

l. Mode of Action of Crystal-forming and Non-crystal-forming Bacteria of the Bacillus cereus, B. thuringiensis Group. There is good evidence that the crystal produced by B. thuringiensis varieties is a protoxin. Further, the high reducing and alkaline conditions of the larval midgut are capable of dissolving the crystal which is soluble in vitro at pH 9.0 in the presence of thioglycolate. The dissolved crystal protein is attacked by enzymes from dialyzed and centrifuged silkworm anterior midgut material. Experiments are underway to test the by-products of the crystal degradation for toxicity in insects.

Histopathology of the silkworm and other insects indicates that the cell-cementing substances in the host midgut is the substrate susceptible to the crystal toxin. A fully-equipped laboratory to study these cell-cementing substances has been set up during the year. The cementing substances have not been studied in any detail in the past and much basic research into the nature of the material is required. The wax moth is currently being produced in significant quantities to supply bulk, midgut epithelial tissue for extraction of these materials, and a technique for harvesting midgut, in quantity, has been devised.

2. Crystal Preparation and Rurification from <u>Bacillus thuringiensis</u>

<u>Varieties.</u> The separation of toxic crystals from spores in <u>B. thuringiensis</u> and varieties is necessary for basic research with the crystal protein, and for feeding tests to determine susceptibility to the toxin.

Various intricate methods have been devised by other workers to effect this separation with spore-crystal preparations grown in the laboratory. Total cost with such methods is approximately \$25,000 per gram of pure crystal. None of these methods separate spores and crystals from commercial preparations.

A new method developed here, employing a differential centrifugation technique coupled with serial washing of the final pellet, enables the theoretical production of 800 mgms per week with one operator, using commercially-produced bacteria. This method, to be published soon, should provide an adequate supply of pure crystal preparations for all work planned.

- 3. Exotoxins Produced by the Bacillus cereus Group. It has been previously demonstrated that at least one other toxin, Phospholipase C, is produced by all pathogenic species of the B. cereus group, including the crystalformers, varieties of Bacillus thuringiensis and B. finitimus. The crystalformers incapable of producing Phospholipase C, e.g., B. entomocidus and variety, have recently been studied and it has been demonstrated that they cause a breakdown of lecithin. Since lecithin is a vital fat, its destruction in the cell is undoubtedly fatal to the insect. As a result of these findings 52 species of the B. cereus group have been taken under study and an action against lecithin outside of the pH range of the known phospholipases has been detected from B. cereus pathogenic for the codling moth. The possibility of this substance being a new type of phospholipid enzyme is being investigated.
- 4. Clostridial Pathogens of Insects. Recently several species of anaerobic bacteria have been isolated in Canada from dead tent caterpillar larvae. Feeding tests indicated that Malacosoma pluviale and M. americanum are susceptible to these pathogens. These bacteria are extremely fastidious in their growth habits. However, limited growth can be obtained in tryptose phosphate broth, with cysteine as a reducing agent, when an alkaline extract of apple leaves is added. Sporulation may be possible in modifications of the above medium, but the empirical nature of the mixture does not allow reproducible results. Accordingly, studies of defined media to permit good growth are in progress. These bacteria are unusual in that they grow only in the gut of the insect and never invade the body cavity. No toxic compound has been isolated, due possibly to the difficulty in growing the bacteria in artificial media.
- 5. Pathogenic Strains of Serratia marcescens. Cultures of pathogenic S. marcescens have been obtained from various sources. The cultures have been, in some cases, extensively tested against insects by other workers, and had previously been thought to be pure. Our studies have shown that these cultures are not pure strains but consist of populations of genetically stable clones differing in colony and cellular morphology. Further, consistently different toxin and enzyme production was recorded for the various clones. Some of the separated clones were slightly pathogenic for salt-marsh caterpillar larvae. However, none of the bacteria multiplied extensively, if at all, in the gut of these insects.

6. Effect of High Altitude Radiation on Spores of Insect Pathogens. Studies to determine the effects of high altitude radiation, particularly heavy cosmic primaries, on the gain or loss of pathogenicity and other changes in the biochemistry of the insect pathogenic bacteria, Bacillus popilliae and B. thuringiensis, have been undertaken in cooperation with the U. S. Naval Missile Center. Protocols for tests were submitted and approved for two of the three mission profiles requested, recoverable probes to an altitude of 250,000 feet and recoverable space probe into the Van Allen Belt, as a part of the U. S. Navy Papoose Program.

To satisfy the first requirement, arrangements were made to incorporate the biopacks into the BUWEPS Hugo III Project, a recoverable photographic reconnaisance probe powered by a Terrier-Cajun two-stage rocket. Apogee 400,000 feet, with payload proceeding downrange 80 miles for recovery. Estimated time above 100,000 feet about 350 seconds. Seven flights with biopacks mated to each payload were scheduled, to be launched from Point Mugu, Calif. Dummy packages were submitted to the Physical Science Officer, Rocket Section, Physical Science Laboratory of the New Mexico State University, and these passed the mechanical tests (spin, vibration, axial acceleration) and were mated to the camera payload for rebalancing. The dummies were replaced at the launching site with the biopacks of identical size and weight (1" \times 3" \times 3/8", 49 grams). Three payloads were successfully launched but were not recovered due to failure of the recovery systems, and after the third failure, the remaining flights were cancelled to permit modification of the design. The failure to recover the payloads and mated biopacks prevented fulfillment of the study, but some information of value in the conduct of the tests was obtained. Processing nuclear emulsion plates returned from unflown payloads showed that the special clamps designed to permit development of the plates without damage to the water dispersible spore layer deposited on the reverse side of the plate were satisfactory. The method of development, fixing, and drying the nuclear emulsion did not cause distortion that would interfere with accurate alignment of tracks to the spores. Aedes aegypti eggs held in the packages for 45 days hatched as well as the laboratory controls, indicating that the amount of oxygen provided in the package was adequate. The Aedes eggs were included in the biopack to obtain some preliminary information in respect to a similar proposal submitted by a cooperating entomologist requesting payload space in recoverable probes into the Van Allen Belt.

Additional sets of biopacks were prepared and installed in the four remaining payloads in the series, but due to technical difficulties with drop tests that were to precede actual launching, none were fired. Studies of laboratory control biopacks and of returned confined biopacks indicate that the conditions in the biopacks are adequate to maintain large numbers of eggs in good condition for several months. Eggs in the biopacks for 3 months gave good hatch, equal to that of unpackaged eggs, and after 4 months about 10% hatched, about half that of laboratory controls. These tests indicate that the thin rubber membranes used to waterproof the biopack and retain its atmosphere is sufficiently porous to permit slow diffusion of carbon dioxide out of the biopacks and sufficient oxygen to enter so that eggs remain viable for longer periods than would have been possible had this diffusion not occurred.

- C. Nematodes and Their Associated Bacteria Pathogenic to Insects.
- 1. <u>DD-136 Nematode for Control of Codling Moth</u>. Field tests were continued in one apple orchard in West Virginia and two orchards in Indiana, in cooperation with entomologists of the Fruit and Vegetable Insects Research Branch, to determine whether applications of the DD-136 nematode would provide effective control of the codling moth. The nematodes used in these tests were produced at this laboratory and transported or shipped to the field. The nematode applications were made in the fall of 1960 and in the spring and summer of 1961. The Insect Pathology Laboratory was responsible for studying the effects of the treatment on the larvae. The cooperating research branch determined the effects on infestation trends in fruit.

In the Kearneysville, W. Va., orchard the number of overwintering larvae that survived under bands was reduced to 0.18 per band before pupation in the spring, a minimum reduction of 98%. Bark scrapings above the bands indicated that the effectiveness of the nematodes was not limited to the area covered by the bands, but extended to the entire trunk of the tree at least. The minimum reduction of overwintering larvae under bands was 98.7% in the Vincennes, Ind., orchard before pupation in the spring. In the Linton, Ind., orchard, where drought conditions occurred when the nematodes were applied in the fall and extended to cold weather, the reduction of overwintering larvae prior to pupation was 76.5%. The high degree of mortality of the larvae indicated that DD-136 nematode sprays thoroughly applied are highly effective against larval populations. Even though the nematode applications were effective in causing high reductions of overwintering larvae, adequate prevention of codling moth damage to the fruit was not attained in any of the test orchards. The reasons for the lack of close correlation between indicated reductions in larval populations and reductions in fruit infestations require

further investigation. Substantial survival of larvae on upper limbs of the trees which were not sprayed, survival of larvae on ground trash and other vegetation, and lack of sufficient isolation which permitted migration of moths from other orchards to the test plots, are some of the factors that may have contributed to the higher than expected fruit infestations.

2. <u>Susceptibility of Aedes egypti to DD-136 Nematode</u>. Tests of the effect of the DD-136 nematode on <u>A. egypti</u> showed that larvae, pupae, and adults are highly susceptible to infection.

Second, third, and fourth stage <u>Aedes</u> larvae exposed on moist filter paper are quickly penetrated by the nematode larvae and are literally torn apart within 30 minutes after exposure. Pupae and adults do not show this effect but are killed within 20 hours. The nematode produces small fertile adults in this host.

D. General

Monitoring Electrophysiological Signals and Locomotor Activity of Insects. (In Cooperation with Pesticide Chemicals Research Branch.) Relatively simple electronic circuits were developed and used to amplify, integrate, and record the signals obtained from silver electrodes inserted in the coxa of the rear legs of the Madeira roach, Leucophaea maderae, under constant light, and normal day-night conditions. These signals could be monitored and recorded continuously for a month or more, and showed bursts of activity during night periods that gave indication of circadial rhythm. Locomotor activity was detected by a capacity-sensing device and the output was recorded for long periods. No attachments to the insect were required and the detector did not interfere with the normal activity of the insect. Protocols for employing these techniques in space probes and satellites to study the effect of extraterrestial environments on insect rhythms have been prepared and submitted to appropriate agencies. Several papers on the subject have been accepted for publication and are now in press.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Virus Diseases of Insects

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AREA 23. INSECT PHYSIOLOGY AND MODE OF ACTION OF INSECTICIDES AND THEIR METABOLITES

Problem. Basic research in insect physiology is essential to the development of more efficient insecticides and new approaches to insect control. The increasing development of resistance to insecticides by insects has emphasized the need for additional information on the mode of action and metabolism of insecticides in insects and the mechanisms of the resistance to insecticides. More knowledge is also needed on the normal physiology and biochemistry of insects to permit a comparison and interpretation of the data obtained from studies on insect toxicology. Basic research in insect biochemistry and physiology, including insect nutrition, will provide a better understanding of the biochemical and physiological systems which regulate insect growth, metamorphosis, reproduction, and diapause, and the chemistry and action of the hormones which mediate these systems. Knowledge gained from such research is essential to the development of new methods of effective insect control which are safer and more selective in their action than the methods now being used. More basic information on the response of insects to light, sound, food, and sex attractants could contribute to better insect control. Insects are useful test animals for basic physiological studies on life processes.

USDA PROGRAM

The <u>Department</u> has a continuing long-term program involving insect physiologists, biologists and chemists engaged in basic studies in insect physiology and biochemistry and in the mode of action of insecticides and their metabolites. At the Pioneering Research Laboratory on Insect Physiology at Beltsville, Md., basic research is conducted on <u>insect resistance to insecticides</u>, <u>biochemistry and physiology of lipids in insects</u>, <u>nutrition and hormones</u>, and <u>effects of light on insect growth and development</u>. Certain aspects of the lipid work are in cooperation with scientists at the National Institutes of Health, Bethesda, Md.

The <u>Federal</u> scientific effort devoted to research in this area totals 10 professional man-years. Of this number 4.0 is devoted to the <u>biochemistry</u> and physiology of lipids in insects, 4.0 to <u>insect nutrition</u> and hormones, and 2.0 to the <u>effects of light on insect growth</u>.

Additional research in this area is provided by the following P. L. 480 projects: S 5-ENT-3 Columbia (2 professional man years); A 7-ENT-6 India (2 professional man years); E 21-ENT-3 Poland (1 professional man year); and E 21-ENT-4 Poland (1 professional man year).

State Experiment Stations in 1961 reported a total of 30.1 professional man-years divided among subheadings as follows: Mode of action and metabolism of insecticides in insects 15.4; insect resistance to insecticides 8.1; biochemistry and physiology of lipids in insects 3.5; insect nutrition and hormones 2.4; and effect of light and sound on insect growth and development 0.7.

Industry and other organizations also conduct research on insect physiology and biochemistry, the mode of action of insecticides and their metabolites, and resistance of insects to insecticides, with special emphasis on the specific compounds that they have developed or intend to develop commercially. Estimated annual expenditures are equivalent to approximately 10 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Insect Resistance to Insecticides

1. Comparing Resistance of House Flies to the Eight Isomers of Allethrin. The toxicity of the eight isomers of allethrin to susceptible and pyrethrin-resistant flies was measured. Toxicity involved both optical and geometric specificity with the asymmetric site on the chrysanthemumate apparently dominant. Flies selected with pyrethrins developed highest resistance to the least toxic allethrin isomers. Dosage-response slopes were not parallel for all isomers and showed no change correlated with resistance.

Results of this study have been presented in the following paper:

Barker, Roy J. and Edmunds, L. N., Jr. Comparing resistance of house flies to the eight stereoisomers of allethrin. Accepted for publication in Jour. Econ. Ent.

B. Biochemistry and Physiology of Lipids in Insects

l. Metabolism of H^3 - β -Sitosterol by House Fly Larvae. As a continuation of work on the utilization and fate of plant sterols in insects, the metabolism of H^3 - β -sitosterol was studied in house fly larvae. The adult of this insect has previously been found to lack the biochemical mechanism for dealkalating the side chain of this C-29 sterol to form cholesterol. House fly larvae were reared aseptically on a semi-defined diet containing 0.1% or 0.2% H^3 - β -sitosterol. Of the total H^3 compounds present in the pupae, more than 97% was free sterols and less than 1% was sterol esters. Only 0.1% behaved as 5,7-dienes when the free sterols were analyzed by column chromatography. The major portion of the radioactivity (98%) was eluted in the Δ^5 fraction. When this fraction was further analyzed by reverse isotope dilution and gas-liquid chromatography, the major H^3 compound was found to be unchanged β -sitosterol, and no conversion of β -sitosterol

to cholesterol was detected. Thus the larva of this insect can also use β -sitosterol per se to fulfill, at least in part, its sterol requirements.

The very small amounts of both the sterol esters and $\Delta^{5,7}$ -dienes found in the pupae, as opposed to the higher concentrations previously found in the adult and/or eggs, suggests a difference in the utilization and roles of these metabolites in different developmental stages of the fly.

- 2. Dietary Sterols and Reproduction in the Male House Fly. A dietary source of sterol is required for sustained viable egg production in the female house fly. To determine if a dietary source of sterol is also necessary for the normal reproductive processes of the male house fly, replicate groups of flies were held for approximately 12 days on semi-defined diets with and without cholesterol. The males from these groups were placed in cages with an equal number of 1-dayold virgin female flies. After 3 days the males were removed and the females were fed on a semi-defined diet containing cholesterol and their eggs were collected. No significant difference was detected in either the total egg production or the egg hatch between those eggs from females inseminated by the males fed on a sterol deficient diet or on a diet containing sterols. This indicates that either a dietary source of sterol is not essential for normal reproduction in the male, or that sufficient sterol is stored from the larval stage to permit the adult male to carry out these processes in the absence of dietary sterol.
- 3. Sterol Esters of House Fly Eggs. It has been found with both H^3 - β -sitosterol and C^{14} -cholesterol that the house fly egg is unique in that it is the only developmental stage of this insect that contains a high percentage of sterol esters. Because of the importance of these sterols in the eggs for normal embryonic development and hatch, the fatty acid moiety of the sterol esters was investigated.

Flies were reared on semi-defined larval and adult diets in which the only added lipid component was C^{14} -cholesterol. The eggs from these flies were collected and the total lipids isolated by chloroform-methanol extraction. Analysis of these lipids by column chromatography showed that about 42% of the C^{14} sterols present were esterified. This is higher than that found previously with either injected C^{14} -cholesterol or dietary H^3 - β -sitosterol.

The sterol esters were transesterified in methanol and the fatty acid methyl esters from the sterol ester fraction were analyzed by gasliquid chromatography. These were primarily (90%) mono-unsaturated compounds up to C-18 in chain length. These findings were confirmed by chromatographing the hydrogenated methyl esters.

For comparative purposes the triglyceride fractions from the eggs and adult flies were also analyzed by the same techniques. The fatty acids of this fraction were almost identical in both the eggs and the flies, and were qualitatively similar to the fatty acids of the egg sterol esters. However, the triglyceride fractions differed quantitatively from the sterol esters in that they contained more than 40% saturated fatty acids.

It appears then, as in certain mammalian systems, that the sterol ester fraction of house fly eggs contains a high percentage of unsaturated fatty acids. However, the multiple unsaturated long-chain fatty acids, so characteristic of the sterol esters of many mammals, were not present in appreciable amounts in the house fly egg sterol esters.

Biochemical Studies with 4-Cl4-Cholestanol in the German cockroach. The German cockroach is known to modify the side chain of certain C-28 and C-29 sterols prior to or during utilization. To determine if this organism also has available the enzymic mechanism for altering the ring structure of a saturated sterol, newly hatched German cockroaches were fed on a semi-defined diet containing 0.2% Cl4-cholestanol plus a subminimal amount of Δ^5 -sterol. When about 20% of the cockroaches had reached the adult stage, they were killed and the total Cl4 compounds extracted. Fractionation of these total extracts by column chromatography indicated that 46% of radioactive compounds behaved as sterol esters and 43% as free sterols. The predominance of sterol esters over the free sterols differs from that previously found with Cl4-cholesterol for this insect, where only 5 to 6% is converted to esters.

When the free sterols were analyzed further by chromatography on alumina as their acetates, the radioactivity was about equally distributed between two major peaks. When an aliquot of the less polar peak (I), which contains stanols and Δ^5 -sterols, was analyzed by reverse isotope dilution with authentic cholesterol, no conversion of cholestanol to cholesterol was detected. Reverse isotope dilution of this fraction with cholestanol and purification through derivative formation did not result in a decrease in specific activity, indicating that this insect uses cholestanol without conversion to cholesterol. A more polar compound, eluted with peak II, behaved as Δ^{7} cholestenol when analyzed by gas-liquid chromatography and reverse isotope dilution. When a sufficient quantity of this metabolite was isolated from a large number of roaches, both the free sterol and its acetate had the same infrared spectrum, melting point, and relative retention time by several gas-liquid chromatographic systems as authentic Δ' -cholestenol. The significance of this conversion and of the accumulation and function of the metabolite in this insect is not presently understood. However, it bears an analogy to the dehydrogenation of Δ^5 -sterols to form 5.7-dienes, a conversion that has

been reported for the German cockroach and certain other insects.

Results of this research have been presented in the following papers:

Ishii, Shoziro, Kaplanis, J. N., and Robbins, W. E. Distribution and fate of 4-Cl4-cholesterol in the adult male American cockroach. Accepted for publication in Annals Ent. Soc. Amer.

Kaplanis, John N., Monroe, R. E., Robbins, W. E., and Louloudes, S. J. The fate of dietary ${\rm H}^3$ -sitosterol in the adult house fly. Accepted for publication in Annals Ent. Soc. Amer.

Louloudes, S. J., Chambers, D. L., Moyer, D. B., and Starkey, J. H., III. The hydrocarbons of adult house flies. Accepted for publication in Annals Ent. Soc. Amer.

VandenHeuvel, W. J. A., Robbins, W. E., Kaplanis, J. N., Louloudes, S. J., and Horning, E. C. The major sterol from cholesterol fed American cockroaches. Accepted for publication in Annals Ent. Soc. Amer.

C. Insect Nutrition and Hormones

1. Effect of Cholesterol in the Larval Diet on Ovarian Development in the Adult House Fly. The adult house fly has been reported to require a dietary source of both protein and carbohydrate for ovarian maturation and to show little or no ovarian growth on an adult diet consisting solely of carbohydrates. This requirement of the adult for protein could not be fulfilled by supplementing the larval diet with additional protein.

In the course of investigations on nutrition and reproduction in the house fly, it was observed that at six days after emergence about one-half of the adult flies reared on CSMA larval medium supplemented with 1% or 2% cholesterol developed mature ovaries when held on an adult diet of only sucrose and water. Only about 2% of the control flies reared on CSMA medium not supplemented with cholesterol showed ovarian development. Thus, under these experimental conditions, supplementing the larval diet with cholesterol resulted in ovarian maturation even in the absence of a source of protein in the adult diet. These results were confirmed with four other laboratory strains of house flies.

This ovarian maturation occurred in the absence of any mortality, which indicated that it was not brought about by the female flies feeding on dead flies as has previously been reported. In addition, eggs from these flies were viable and produced larvae which developed to adults.

To determine whether or not the activity observed was brought about by micro-organisms present in the larval medium, the tests were repeated with a synthetic larval diet and aseptic rearing technique. For comparison, the phytosterol β -sitosterol, which is one of the major sterols present in the CSMA medium, was also tested. From 25% to 68% of the flies reared aseptically on the synthetic larval diet containing various concentrations of cholesterol and held on an adult diet of sucrose and water contained mature ovaries, whereas only 1% to 5% of those flies reared on the diet containing β -sitosterol showed ovarian development. This lack of equivalence in the biological activity of cholesterol and β -sitosterol is in agreement with the results of prior biochemical studies with the house fly at this laboratory, using tritiated β -sitosterol, in which no conversion of β -sitosterol to cholesterol could be detected.

These results point to an interesting physiological role for cholesterol in the house fly, an insect which has previously been shown to require a dietary source of sterol for growth and development and for sustained viable egg production. Studies are currently underway to determine whether cholesterol as such is responsible for this effect, or whether it serves as precursor for an essential steroid metabolite which regulates gonadal development.

2. <u>Gonadotropic Hormone</u>. Using the previously reported bioassay technique, a number of total extracts and fractions from whole insects and insect tissues have been tested for gonadotropic activity. Certain whole insect and tissue extracts caused a definite positive response when assayed using allatectomized roaches.

Techniques for the fractionation and enrichment of these active extracts and chromatographic systems for purifying and concentrating the hormones are being investigated. Preliminary fractionation of the most active insect extract by two chromatographic systems effected approximately a 50-fold concentration of the active material.

In addition, certain synthetic compounds have been tested. One of these—farnesol—is known to mimic the action of the juvenile hormone in certain insects and to have gonadotropic activity in decapitated adult female Rhodnius. When tested for gonadotropic activity in allatectomized roaches, this compound showed little or no activity when injected in peanut—oil solutions or emulsions with a wide range of doses, the highest of which caused some mortality. However, the topical application of farnesol to the cuticle of the abdomen of allatectomized roaches did cause definite ovarian growth and yolk deposition. Farnesol behaved chromatographically different from the active principles found in the insect extracts.

3. Ecdysone. Improved larval and adult diets have been worked out for <u>Calliphora vicina</u>, the test organism used for the bioassay of the insect molting hormone (ecdysone). These diets facilitate the maintenance of colonies through an increase in total and viable egg production from the adults and a higher yield in both the number and weights of larvae. Larvae reared on the improved diet showed a 50-60% increase in suitable test organisms following ligature.

Active extracts have been obtained from several insects in addition to those previously reported, and fractionation and purification of these extracts is underway to provide highly purified active material for biochemical and physiological studies.

A limited number of synthetic compounds have been tested using the <u>Calliphora</u> bioassay but none of these has given a positive test.

Some of the results of this research have been presented in the following paper:

Monroe, R. E., Robbins, W. E., Chambers, D. L., and Tabor, L. A. Sterol antagonists and house fly reproduction. Accepted for publication in Annals Ent. Soc. Amer.

D. Effects of Light and Sound on Insect Growth and Development

Photoperiodism in Pieris rapae. To study the role of light in diapause inhibition, imported cabbageworms, Pieris rapae, were reared in cans with controlled temperature and photoperiod. At 20° C., but not at 24° C., diapause occurred with a short photoperiod. All larvae diapaused with 10 hours light in a 24-hour cycle; two-thirds diapaused with 12 hours light; none diapaused with 14 or 16 hours light. No critical photoperiod existed near 12 hours for which a short increase in photoperiod gave an abrupt decrease in diapause. Diapause gradually diminished as the light period increased from 11 to 13 hours accompanied by a dark period decrease from 13 to 11 hours. Results from 24-hour cycles suggested that the length of the dark period is more important than the period of light exposure. dark period is interrupted at a critical time, even 30 minutes of light completely prevents diapause. This interrupting light is effective whether it precedes or follows a 10-hour photoperiod by 3 1/2 hours. The fourth instar larvae respond best to the effects of photoperiod, but exposure of any stage to long days gives fewer diapausing pupae than does continuous exposure to short days.

<u>Pieris rapae</u> resembled many other animals in being responsive to a brief nocturnal illumination supplementing a non-effective short diurnal photophase. The eight minutes of light found to be effective for <u>Pieris</u> is the shortest photophase yet found to inhibit diapause in insects.

The results of this study have been presented in the following paper:

Barker, Roy J., Mayer, Ann, and Cohen, C. F. Photoperiod effects in <u>Pieris rapae</u> L. Accepted for publication in Annals Ent. Soc. Amer.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

Biochemistry and Physiology of Lipids in Insects

- Louloudes, Spiro J., Thompson, M. J., Monroe, R. E., and Robbins, W. E. 1962. Conversion of cholestanol to A7-cholestenol by the German cockroach. Biochem. & Biophys. Res. Comm. 8 (2): 104-106.
- Monroe, R. E., Kaplanis, J. N., and Robbins, W. E. Sterol storage and reproduction in the house fly. Annals Ent. Soc. Amer. 54 (4): 537-539.
- Robbins, W. E., Dutky, R. C., Monroe, R. E., and Kaplanis, J. N. 1962. The metabolism of H³-\beta-sitosterol by the German cockroach. Annals Ent. Soc. Amer. 55 (1): 102-104.

Insect Nutrition and Hormones

- Mitlin, Norman. 1962. The composition of ribonucleic acid in several holometabolous insects. Annals Ent. Soc. Amer. 55 (1): 104-105.
- Mitlin, Norman, and Cohen, C. F. 1961. The composition of ribonucleic acid in the developing house fly ovary. Jour. Econ. Ent. 54 (4): 651-653.
- Monroe, R. E. 1962. A method for rearing house fly larvae aseptically on a synthetic medium. Annals Ent. Soc. Amer. 55 (1): 140.
- Robbins, W. E., and Shortino, T. J. 1962. Effect of cholesterol in the larval diet on ovarian development in the adult house fly. Nature 194 (4827): 502-503.

Work &			Line Proj	.Inc. in
Line Project		Work Locations	Summary	Area &
Number	Work and Line Project Titles	During Past Year	Progress	Sub- Heading
ENT bl(R)	Sugar beet insect investigations			nedding
ENT bl-l (R)	Control methods and biological studies of insects and mites affecting sugar beets	Mesa, Ariz.	Yes	11-B-2 11-G-2
		Logan, Utah	Yes	11-B-2
		Twin Falls, Idaho Yakima, Wash.	Yes Yes	11-B-2 11-B-2
		Fort Collins, Colo.	Yes	11-B-2 11-F-2
ENT b2(R)	Tobacco insect investigations			
ENT b2-1 (R)	Biological control methods and biology of insects attacking tobacco foliage	Oxford, N. C.	Yes	10-A-1 10-D-1,2 10-E-1
		Florence, S. C.	Yes	10-A-1 10-D-1
ENT b2-2 (R)	Insecticide control methods for insects attacking tobacco foliage	Quincy, Fla.	Yes	10-A-1
ENT b2-3 (R)	Control methods and biology of soil insects that attack tobacco	Florence, S. C.	Yes	10-B-1
ENT b3(R)	Greenhouse and ornamental plant insects			
ENT b3-1	Biology and methods of control of insects on greenhouse ornamental and vegetable crops	Farmingdale, N. Y. Sumner, Wash.	Yes Yes	12-B-1 12-A-1 12-B-1
		Beltsville, Md.	Yes	12-A-1
				12-B-1
				12-C-1
				12-D-1
ENT b3-2	Insects in relation to diseases of greenhouse and ornamental plants	Beltsville, Md.	Yes	12-F-1
ENT b3-3	Biology and methods of control of insect pests of	Farmingdale, N. Y.	Yes	12-B-1
	outdoor flowers	Sumner, Wash.	Yes	12-B-1
		Beltsville, Md.	Yes	12-B-1
				12-C-1 12-F-1
mem a le				
ENT b4	Vegetable and berry insects			
ENT 64-1	Biology and methods of control of insects and	Riverside, Calif.	Yes	1-B-10
(R)	mites affecting beans and peas	Beltsville, Md. Walla Walla, Wash.	Yes Yes	1-B-10 1-B-2
		Charleston, S. C.	Yes	1-B-2
		0222002, 27 07		
ENT b4-3 (R)	Biology and methods of control of insects affecting melons and other cucurbits	Charleston, S. C.	Yes	1-B-4,8
ENT 04-4	Biology and methods of control of the beet leaf-	Twin Falls, Idaho	Yes	1-B-1,4
(R)	hopper as a pest of vegetables	Logan, Utah	Yes	1-B-1,4
ENT 64-5	Insects in relation to diseases of vegetables	Mesa, Ariz.	Yes	1-H-1
	and berries	Fort Collins, Colo.	Yes	1-A-2
		Yakima, Wash.	Yes	2-A-2 2-G-1
		Touristics, House,	168	
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Work & Line			Line Proj	.Incl. in
Project		Work Locations	Summary	Area &
Number	Work and Line Project Titles	During Past Year	Progress	Sub- heading
ENT b4-6 (R)	Biology, host plant relationships, and methods of control of insects that attack potato	Orono, Me.	Yes	2-A-1 2-B-1
(,	•	Yakima, Wash.	Yes	2-A-1
				2-B-1
ENT 64-7	Methods of preventing deleterious residues	Mesa, Ariz.	Yes	1-B-6
(R)	resulting from the use of insecticides on	Riverside, Calif.	Yes	1-B-6
	vegetables and berries	Charleston, S.C.	Yes	1-A-1
				1-B-5,6 1-C-3
		Beltsville, Md.	Yes	1-A-1
				1-B-6
				1-C-4,5 2-C-1
		Orono, Me.	Yes	2-C-1
		Yakima, Wash.	Yes	2-C-1
TOWN S.J. Q	Towastinations on the was of natural anomaes and	Discountie Calif	Ye c	3 D le
ENT b4-8 (R)	Investigations on the use of natural enemies and other biological methods for the control of	Riverside, Calif.	Yes	1-D-4 1-E-4
(11)	vegetable and berry insects	Fort Collins, Colo.*	Yes	1-E-4
		Twin Falls, Idaho	Yes	1-E-4
		Beltsville, Md.	Yes	1-A-3 1-D-1
				1-E-1,
				2,3
		Walla Walla, Wash.	Yes	1-D-2 1-E-4
		Charleston, S.C.	Yes	1-D-3
				1-E-4
		Orono, Me.	Yes	2-E-1 2-D-1
		Orono, re.	100	~-D-1
ENT 64-9		Beltsville, Md.	Yes	3-B-9
(R)	mites affecting strawberries and bramble berries	Riverside, Calif.	Yes	3- B -9
	Biology and methods of control of insects	Charleston, S.C.	Yes	1-B-7
(c)(R#2)	affecting underground portions of vegetables			2-A-3
				2-B-2
ENT b4-	Improvement of methods and evaluation of equipment	Forest Grove, Oreg.	Yes	1-F-1,2
12 (R)	for applying insecticides to vegetable crops	Charleston, S.C.	Yes	1-F-1
ENT b4-	Development of methods for preventing contamination	Beltsville, Md.	Yes	1-A-4
14	of processed vegetables and berries by field	20200, 2220, 1220		3-A-1
(R#2)	insects			3-E-1
				3-F-4
ENT b5	Methods of treating plants and commodities			
(R)	regulated by plant quarantines			
ENT 15-1	Development of treatments for plants and	Hoboken, N. J.	Yes	4-c-2,3
(R)	commodities regulated by plant quarantines	nosonon, m o	100	4-G-2,3,
				4,5
ENT b6	Mexican fruit fly and other fruit pests in Mexico			
22,12 30	that threaten U. S. horticulture			
	*Station closed in January 1962			

Line Project Check List Reporting Year July 1, 1961 to June 30, 1962				
Work &				. Incl. in
Line		Work Locations	Summary	Area & Sub-
Project	Work and Line Project Titles	During Past Year	Progress	heading
Number				Heaving
ENT b 6-1	Biology, ecology, habits and natural enemies of the Mexican fruit fly and citrus blackfly	Mexico City, Mex.	Yes	4-A-2
ENT b6-2 (R)	Studies of lures for Mexican fruit flies	Mexico City, Mex.	Yes	4-E-2
ENT b 6-3	Insecticide control of Mexican fruit fly	Mexico City, Mex.	Yes	4-B-2 4-E-2
ENT 66-4	Sterilization of products infested by fruit flies in Mexico	Mexico City, Mex.	Yes	4-C-1 4-F-2 4-G-1
ENT b7	Investigations of fruit flies in Hawaii			
ENT b7-1	Ecology and biology of fruit flies and their natural enemies in Hawaii	Honolulu, Hawaii	Yes	4-A-2 4-D-2 4-H-1
		Hilo, Hawaii	Yes	4-A-2 4-D-2
		Kahului, Hawaii	Yes	4-A-2 4-D-2
ENT b7-2	Development of new or improved mass production methods and manipulation techniques for fruit flies and biological control agents	Honolulu, Hawaii	Yes	4-A-1
ENT b7-3	Investigation of fruit fly lures and repellents in Hawaii	Honolulu, Hawaii Hilo, Hawaii Kahului, Hawaii	Yes Yes Yes	4-E-2 4-E-2 4-E-2
ENT b7-4	Chemical methods for control of fruit flies in Hawaii	Honolulu, Hawaii	Yes	4-B-2
ENT b7- 5 (R)	Development of the sterile male release, male annihilation, and other area control and eradication procedures for fruit flies	Honolulu, Hawaii Hilo, Hawaii Kahului, Hawaii Guam*	Yes Yes Yes Yes	4-E-2 4-E-2 4-E-2 4-E-2
ENT b7- 6	Commodity treatments to destroy fruit flies and associated pests of quarantine importance in fresh fruits and vegetables in Hawaii	Honolulu, Hawaii	Yes	4-C-2 4-G-1
ENT b7-7	Ionizing radiation treatments for fruit fly- infested fruits and vegetables	Honolulu, Hawaii	Yes	4-G-1
ENT b8	Deciduous fruit and nut insect investigations			
ENT b8-1 (R)	Studies of the codling moth and its control	Yakima, Wash.	Yes	3-B-1 3-E-1 3-F-1
		Kearneysville, W. Va.	Yes	3-B-1 3-D-1
		Vircennes, Ind.	Yes	3-A-1 3-B-1 3-D-1 3-E-1
ENT b8-2 (R)	Studies of orchard mites and their control	Yakima, Wash.	Yes	3-B-2 3-E-1 3-F-1
	*Station established in February 1962			

	Line Project Check List Reporting Year Jul	y 1, 1961 to June 30, 19	62	
Work &			Line Proj	.Incl. in
Line Project	Work and Line Project Titles	Work Locations During Past Year	Summary of	Area & Sub-
Number ENT b8-2	Studies of orchard mites and their control	Wanatahaa Wash	Progress	heading
(R)	(cont'd.)	Wenatchee, Wash.	Yes	3-B-2 3-E-1
(2.)	(0020 4.)	Kearneysville, W.Va.	Yes	3-B-2
		Vincennes, Ind.	Yes	3-B-2
				3-C-1
		Wooster, Ohio	Yes	3-E-1 3-B-2
		Hooseer, onto	165	2-1-2
ENT b8-3	Studies of the plum curculio and its control	Fort Valley, Ga.	Yes	3-B-3
				3-E-1
		Vincennes, Ind. Wooster, Ohio	Yes Yes	3-B-3 3-B-3
		Moorestown, N.J.	Yes	3-B-3
		1.000 0.000 1.000		3 2 3
ENT b8-4	Studies of borers attacking deciduous fruit	Fort Valley, Ga.	Yes	3-B-4
	trees and their control			3-D-1
		Vincennes, Ind.	Yes	3-E-1 3-B-4
		, , , , , , , , , , , , , , , , , , ,	100	3-E-1
ENT b8-5	Studies of the pear psylla and its control	Yakima, Wash.	Yes	3-A-1
(R)		Wenatchee, Wash.	Yes	3-B-5 3-B-5
				527
ENT b8-6	Studies of miscellaneous insect pests of	Fort Valley, Ga.	Yes	3-B-6
	deciduous fruits and their control	Vincennes, Ind.	Yes	3-A-1
				3-B-6 3-C-1
				3-D-1
		Yakima, Wash.	Yes	3-A-1
				3-B-6
		Manatahan Mash	Yes	3-C-1 3-A-1
		Wenatchee, Wash.	Yes	3-B-6
				3-E-1
		Kearneysville, W.Va.	Yes	3-A-1
				3-B-6
				3-C-1 3-D-1
		Wooster, Ohio	Yes	3-B-6
				3-E-1
ENT b8-7	Investigations of nut insects and their control	Albany, Ga.	Yes	3-B-7
EMI DO-	Threse cigations of mat insects and cheff control	Albany, Ga.	Tes	3-E-1
				3-F-2
i		Shreveport, La.	Yes	3-B-7
		Montdoello Ele X	Vec	3-F-2
		Monticello, Fla.*	Yes	3-A-1 3-B-7
				3-E-1
		Vincennes, Ind.	Yes	3-E-1
ENT 58-8	Grape insect investigations	Wooster Ohio**	Yes	2 7 8
ENT DO-O	Grape insect investigations	Wooster, Ohio**	I Tes	3- B- 8,
				3-C-1
				3-F - 3
	*Station closed in September 1962			
	**Transferred from Sandusky, Ohio, in			
	January 1962			
			1	

Line Project Check List - - Reporting Year July 1, 1961 to June 30, 1962

	Line Project Check List Reporting Year	July 1, 1901 to June 30		
Work &				j. Incl. in
Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Summary of Progress	Area & Sub- heading
ENT b9(R)	Investigations of insect and mite vectors of deciduous tree fruit viruses	Fort Valley, Ga.	Yes	3-B-8
ENT b9-1 (R)	Distribution of insects and mites in and near deciduous fruit orchards infected with virus diseases	Riverside, Calif. Fort Valley, Ga.	Yes Yes	3-G-2,5 3-G-1
ENT b9-2	Studies of insect vectors of phony peach virus disease and their control	Fort Valley, Ga.	Yes	3-A-2 3-G-1
ENT b9-3 (R)	Studies of mite vectors of peach mosaic virus disease, including biology, ecology, and control	Riverside, Calif.	Yes	3-A-2 3-G-2
ENT b9-4 (R)	Transmission studies with possible insect and mite vectors of the latent group of stone fruit viruses	Riverside, Calif. Corvallis, Oreg. Madison, Wis.*	Yes Yes Yes	3-G-3 3-G-3 3-G-3
ENT b9-6	Studies on the biology and control of mite vectors of peach mosaic	Riverside, Calif.	Yes	3-G-2
ENT b9-7	Transmission studies with possible insect, mite, and nematode vectors of miscellaneous stone fruit virus diseases	Riverside, Calif. Fort Valley, Ga. Wenatchee, Wash. Corvallis, Oreg.	Yes Yes Yes Yes	3-G-4 3-G-4 3-G-4 3-G-4
епт ъ9-8	Studies of possible insect and mite vectors of pear decline and their control	Riverside, Calif.	Yes	3-G-5
ENT blo	Insects of citrus and other subtropical fruits			
ENT blo-1	Biology and methods of control of citrus mites	Orlando, Fla.	Yes	4-A-1 4-B-1 4-F-1
		Riverside, Calif.	Yes	4-A-1 4-B-1 4-E-1
ENT b10-2	Biology and methods of control of scale insects, whiteflies and mealybugs on citrus	Orlando, Fla.	Yes	4-A-1 4-B-1
		Riverside, Calif.	Yes	4-A-1 4-B-1
ENT 610-3	Biology and methods of control of miscellaneous insects on citrus and other subtropical fruits	Riverside, Calif. Honolulu, Hawaii	Yes Yes	4-B-1 4-A-3 4-E-3
		Mexico City, Mex.	Yes	4-A-3
ENT blo-4 (R)	Insect vectors of tristeza and other diseases of citrus	Orlando, Fla.	Yes	4-I-1
ENT bl0-5	Investigations of the biological control of citrus insects and mites	Orlando, Fla. Lake Alfred, Fla. Riverside, Calif.	Yes Yes Yes	4-D-1 4-D-1 4-D-1
	*Station closed in June 1962 **Superseded by ENT b9-3 (R) in 1962			

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Work &				
Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Summary Of Progress	Area & Sub- heading
ENT bll	Japanese beetle, European chafer, and related species			
ENT bll-1	Investigations of insecticides for control of the Japanese beetle	Moorestown, N.J.	Yes	12-B-2
ENT bll-2	Development and improvement of treatments to permit movement of nursery stock and farm products under quarantine regulations	Moorestown, N.J.	Yes	12-G-1,2
ENT bll-3	Development of methods of making biological assays of insecticidal residues in soils	Moorestown, N.J.	Yes	12-B-2 12-G-1,2
ENT b11-4 (R)	Investigations of survey methods and biological and chemical control of the European chafer	Geneva, N.Y.	Yes	12-B-3 12-C-3 12-D-2 12-E-1
ENT b11-6	Ecology, biology, and natural control of the Japanese beetle	Moorestown, N.J.	Yes	12-C-2
ENT cl	Boll weevil investigations			
ENT cl-1	Ecology, life history, distribution and damage	Florence, S.C.	Yes	9-A-1
	of the boll weevil	State College, Miss.	Yes	9-A-1
		Stoneville, Miss.	Yes	9-A-1
		Tallulah, La.	Yes	9-A-1
		Waco, Tex.	Yes	9 -A -1
ENT cl-2	Development of effective and economical methods of controlling the boll weevil	Florence, S.C.	Yes Yes	9-B-1 9-D-1
			Yes	9-E-1
		State College, Miss.	Yes	9-D-1
		Stoneville, Miss.	Yes	9-B-1
			Yes Yes	9-E-1
		Tallulah, La.	Yes	9-G-1 9-B-1
		Tarruran, La.	Yes	9-D-1
			Yes	9-E-1
		Waco, Tex.	Yes	9-B-1
		Brownsville, Tex.	Yes	9-E-1
		,	Yes	9-G-1
		College Station, Tex.	Yes	9-B-1
			Yes	9-D-1
			Yes	9-E-1
ENT cl-3	Physiological, nutritional and biochemical	State College, Miss.	Yes	9-A-1
	research on the boll weevil	Baton Rouge, La.	Yes	9-A-1
		College Station, Tex.	Yes	9-A-1
		Florence, S.C.	Yes	9 -A- 1
ENT c2	Bollworm investigations			
ENT c2-1	Biology, ecology and methods of controlling the	Brownsville, Tex.	Yes	9-A-3
	bollworm and other lepidopterous larvae that	1	Yes	9 - D-3
	cause a similar type of injury to cotton		Yes	9-G-2
		State College, Miss.	Yes	9-B-3
		Tucson, Ariz.	Yes	9-D-3

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Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Summary of Progress	Area & Sub- heading
ENT c3	Cotton insects other than boll weevil, bollworm, and pink bollworm and insects attacking other fiber plants			
ENT c3-1	Biology, ecology and methods of controlling miscellaneous insect and spider mite pests	Stoneville, Miss.	Yes Yes	9-B-3 9-E-3
	of cotton	Brownsville, Tex.	Yes Yes	9-A-3 9-C-1
		Tucson, Ariz.	Yes Yes Yes	9-F-1 9-D-3 9-E-3
		Waco, Tex.	Yes Yes	9-G-2 9-B-3
ENT c4	Pink bollworm investigations			
ENT c4-1	Chemical control of the pink bollworm	Brownsville, Tex.	Yes	9-B-2
ENT c4-2	Biological control of the pink bollworm	Brownsville, Tex.	Yes Yes	9-D-2 9-E-2
ENT c4- 3	Cultural, physical and mechanical methods for control of the pink bollworm	Brownsville, Tex. Waco, Tex.	Yes Yes	9-B-2 9-F-1
ENT c4-4	Plant resistance and hosts of the pink bollworm	Brownsville, Tex. Waco, Tex.	Yes Yes	9-A-2 9-A-2
ENT c4-5	Ecological investigations on the pink bollworm	Brownsville, Tex. Waco, Tex.	Yes Yes	9-A-2 9-A-2
ENT c4-6	Physiological, biochemical and morphological studies of the pink bollworm	Brownsville, Tex.	Yes	9-A-2 9-E-2
ENT c4-7	Effects of the pink bollworm and other cotton insects on quality of cotton lint and seed		No	
ENT c5	Corn insects			
ENT c5-1	Biology and ecology of the European corn borer	Ankeny, Iowa	Yes	7-A-1 7-E-1 7-F-1
ENT c5-2	Chemical control of the European corn borer	Ankeny, Iowa	Yes	7-B-1 7-C-1
ENT c5-3	Plant resistance to the European corn borer	Ankeny, Iowa	Yes	7-G-1
ENT c5-4	Biological control of the European corn borer	Ankeny, Iowa	Yes	7-D-1
ENT c5-5	Biology, ecology, and methods of control of the	Tifton, Ga.	Yes	1-G-1 7-A-1
	GOTH EALWOLD	West Lafayette, Ind.	Yes	1-G-1 7-B-1
		State College, Miss.	Yes	1-B-9 7-G-1
ENT c5-6	Biology, ecology and methods of controlling miscellaneous insects attacking corn	Stillwater, Okla. State College, Miss. Tifton, Ga.	Yes Yes Yes	7-A-1 7-B-1 7-G-1

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Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962 Line Proj. Inc. in Work & Summary Line Work Locations Area & Work and Line Project Titles During Past Year of Sub-Project heading Progress Number Plant resistance of corn to rice weevil attack ENT c5-7 State College, Miss. Yes 7-G-1 ENT c5-8 Biology, ecology and methods of control of soil State College, Miss. Yes 7-B-1 insects attacking corn ENT c6 Small grain insects ENT c6-1 Biology, ecology and methods of control of Stillwater, Okla. Yes 7-A-2 7-B-2 aphids attacking small grains 7-G-2 ENT c6-2 Biology, ecology and methods of control of mites Manhattan, Kans. Yes 7-A-2 attacking small grains ENT c6-3 Biology, ecology and methods of control of Manhattan, Kans. Yes 7-A-2 hessian fly and wheat jointworm attacking West Lafayette, Ind. Yes 7-G-2 small grains ENT c6-4 Biology, ecology and methods of control of the Bozeman, Mont. Yes 7-A-2 Minot, N.D. wheat stem sawfly Yes 7-B-2 7-E-2 7-G-2 ENT c6-5 Biology, ecology and methods of control of Stillwater, Okla. 7-A-2 Yes insects attacking sorghums ENT c6-6 Biology, ecology and methods of control of soil No insects and related pests of small grains ENT c6-7 Manhattan, Kans. Distribution, biology, ecology and control of 7-H-1 Yes 8-A-1 (R) insect and mite vectors of small grain Baton Rouge, La. Yes diseases 8-B-1 8-c-1 ENT c6-8 8-A-2 Biology, ecology and methods of control of rice Baton Rouge, La. Yes field insects ENT c7 Sugarcane insects Biology, ecology, and methods of control of 11-A-1 ENT c7-1 Houma, La. Yes borers attacking sugarcane 11-B-1 11-E-1 11-F-1 Canal Point, Fla. Yes 11-D-1 11-E-1 ENT c7-2 Biology, ecology, and control of insects other Houma, La. Yes 11-B-1 than borers attacking sugarcane Canal Point, Fla. 11-B-1 Yes 11-D-1 Biology, ecology, and methods of control of ENT c7-3 Yes 11-G-1 Houma, La. insect and mite vectors of sugarcane diseases ENT c8 Legume and grass insects ENT c8-1 5-D-4 Biology, ecology and methods of control of Yes Mesa, Ariz. (R)(C) 5-G-1,2, insects attacking alfalfa and clovers 4,5 5-G-1 Tucson, Ariz.* Yes 5-G-1 Yes Bakersfield, Calif.* *Research at Bakersfield, Calif., transferred to Tucson, Ariz., in January 1962.

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Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Summary of Progress	Area & Sub- heading
ENT c8-1 (R)(C)	Biology, ecology and methods of control of insects attacking alfalfa and clovers (cont'd.)	Beltsville, Md.	Yes	5-A-2 5-B-2 5-D-3 5-G-3
		Lincoln, Nebr.	Yes	5-A-2 5-A-3 5-B-3 5-G-2,6
		Forest Grove, Oreg.	Yes	5-A-3 5-B-3
		University Park, Pa.	Yes	5-A-3
ENT c8-2	Biology, ecology and methods of control of insects attacking legumes other than alfalfa	Tifton, Ga.	Yes	5-B-4 6-B-1
	and clovers	Columbia, Mo.	Yes	6-A-1 6-B-1
ENT c8-3	Biology, ecology and methods of control of insects attacking grasses	Tifton, Ga.	Yes	5-A-4 5-B-4,5
	and the second s	University Park, Pa.	Yes	5-A-4
ENT c8-4	Insect vectors of pathogenic agents affecting legumes and grasses	Tifton, Ga. University Park, Pa.	Yes Yes	5-H-1 5-H-2
ENT c8-5	Insecticide residues on forage crops	Tifton, Ga.	Yes	5-c-1,2, 3 6-c-1
ENT c9	General feeder insects			
ENT c9-1	Biology, ecology and biological methods of control of armyworms and cutworms	Baton Rouge, La.	Yes	5-D-2
ENT c9-2	Biology, ecology and methods of control of grasshoppers	Mesa, Ariz.	Yes	5-A-1 5-D-1 5-E-1
		Bozeman, Mont.	Yes	5-A-1 5-B-1 5-E-1
		Columbia, Mo.	Yes	5-D-1
ENT c9-3	Biology, ecology and methods of control of white-fringed beetles	Florala, Ala.	Yes	5-B-6 5-E-2
ENT cl0	Bee culture investigations			
ENT cl0-1	Biology of diseases and pests of honey bees and development of control methods	Beltsville, Md.	Yes	18-A-1, 2,3,5, 7,8
		Baton Rouge, La.	Yes	18-A-4, 5,7
		Laramie, Wyo.	Yes	18-A-2, 5,6,7,
		Madison, Wis.	Yes	8,9 18-A-5
ENT cl0-2	Biology, breeding, and management for improvement in productivity of honey bees	Baton Rouge, La. Logan, Utah Madison, Wis. Tucson, Ariz.	Yes Yes Yes Yes	18-B-1 18-B-2 18-B-1,2 18-B-2

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Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Summary of Progress	Area & Sub- heading
ENT cl0-3	Behavior and utilization of honey bees in the pollination of agricultural crops	Tucson, Ariz. Baton Rouge, La. Madison, Wis. Logan, Utah Laramie, Wyo.	Yes Yes Yes Yes Yes	18-C-1,2,5 18-C-3 18-C-3 18-C-4 18-C-4
ENT cl0-4	Biology and utilization of insects other than honey bees in the pollination of agricultural crops	Logan, Utah	Yes	18-D-1
ENT cl0-5	Effects of pesticides and other farm practices on honey bees and other pollinating insects	Beltsville, Md. Logan, Utah Madison, Wis. Tucson, Ariz.	Yes Yes Yes Yes	18-E-1 18-E-1,4 18-E-2 18-E-2,3,4
ENT hl	Mosquitoes, sand flies, and gnats investigations			
ENT hl-l (R#2)	Development of more effective insecticides and other materials and methods for controlling mosquitoes	Reno, Nev.* Fresno, Calif.*	Yes Yes	13-A-1 13-A-1 14-A-2 16-A-2
		Corvallis, Oreg.	Yes	13-A-1 13-B-1 13-E-1 14-A-2 14-B-2 14-E-2 16-A-2 16-B-2 16-C-2 17-B-1
		Orlando, Fla.	Yes	17-D-1 14-A-2 14-B-2 16-A-2 16-B-2 16-C-2 17-B-1 17-C 17-D-1
ENT hl-4 (R#2)	Studies on the distribution, abundance, taxonomy, and biology of mosquitoes affecting	Reno, Nev.*	Yes	13-A-1 17-A-1
(1472)	agriculture	Fresno, Calif.*	Yes	13-A-1 14-A-2 16-A-2
		Corvallis, Oreg.	Yes	17-A-1 13-A-1 14-A-2 16-A-2
		Orlando, Fla.	Yes	17-A-1 14-A-2 16-A-2 17-A-1
	* Research at Reno, Nev. transferred to Fresno, Calif. in September 1961			
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Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Summary of Progress	Area & Sub- heading
ENT hl-5 (R#2)	Development of repellents and other materials and methods to protect man and animals from	Corvallis, Oreg.	Yes	13-B-1 14-B-2
	mosquitoes, sand flies and gnats	Orlando, Fla.	Yes	17-D-1 17-D-1
ENT hl-15 (R)	Studies on the relationship of water and land management procedures to mosquito breeding	Reno, Nev.*	Yes	13-A-1 17-A-1
(11)	in water impoundments and in irrigated farming areas	Fresno, Calif.*	Yes	13-A-1 14-A-2 16-A-2 17-A-1
		Corvallis, Oreg.	Yes	13-A-1
ENT hl-16 (R#2)	Studies on the biology and control of black flies, sand flies, and other gnats and their	Kerrville, Tex.	Yes	15-B-4 15-G-1
V-41	relationship to disease transmission, especially on livestock and poultry	Orlando, Fla.	Yes	17-A-4 17-D-6
		Denver, Colo.**	Yes	15-E-1
ENT h2	Investigations of flies affecting man and livestock			
ENT h2-1 (R#2)	Development of insecticides, repellents and other materials and methods for the control of horn flies, stable flies, and face flies	Lincoln, Nebr.	Yes	13-A-2,3 13-B-3 13-D-3 13-E-3 14-A-3,4 14-B-4 14-D 14-E-4
		Corvallis, Oreg.	Yes	13-A-2,4 14-A-5
		Kerrville, Tex.	Yes	13-A-4 13-B-2 13-E-2 14-A-5 14-B-3,5 14-C-2 14-E-3
		Stoneville, Miss.	Yes	14-B-5
		Orlando, Fla. Beltsville, Md.	Yes Yes	14-E-3 14-E-3
ENT h2-5 (R#2)	Development of improved larvicides and other materials and methods for the control of screw-worms and fleeceworms	Kerrville, Tex.	Yes	13-A-5 13-B-5 13-E-4 14-A-6 14-B-6 14-E-5 15-B-1 15-E-1
ENT h2-7 (R#2)	Studies of irradiation and radioactive insecticides on flies and other arthropods affecting man and animals * Research at Reno, Nev. transferred to Fresno, Calif. in September 1961 **Station established in June 1962	Kerrville, Tex.	Yes	13-A-5,10 13-E-4,6 14-A-6,10 14-E-5,6 15-A-1 15-E-2,3

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Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Summary of Progress	Area & Sub- heading
ENT h2-7 (R#2)	Studies of irradiation and radioactive insecticides on flies and other arthropods affecting man and animals (cont'd.)	Corvallis, Oreg.	Yes	13-A-6 13-E-1,5 14-A-1 14-A-10 14-E-1,2 16-C-1
		Orlando, Fla.	Yes	17-A-1,2 13-E-5 14-E-1 16-C-1 17-A-2 17-D-2
ENT h2-9 (R#2)	Development of insecticides, attractants, and other materials and methods for the control of house flies and blow flies	Corvallis, Oreg.	Yes	13-A-6 13-E-5 14-A-1 14-E-1 16-A-1 17-A-2 17-D-2
		Orlando, Fla.	Yes	13-B-6 13-E-5 14-A-1 14-B-1
				14-E-1 16-A-1 16-B-1 16-C-1 17-A-2 17-B-2 17-D-2
ENT h2-11 (R#2)	Studies on the biology and control of horse flies and deer flies as they relate to pests of animals and vectors of disease	Stoneville, Miss.	Yes	13-A-8 13-B-8 14-A-8 14-B-8
		Kerrville, Tex. Corvallis, Oreg.	Yes Yes	13-G-1 14-G-1
ENT h2-14 (R)	Development of repellents and other methods to protect man from horse flies, deer flies, and stable flies		No	
ENT h2-15 (R)	Development of improved media and mass rearing and distribution techniques for screw-worm control	Kerrville, Tex.	Yes	13-A-5 13-E-4 14-A-6 15-A-1 15-E-1
ENT h2-16 (R)	Development of attractants and other materials and methods for estimating and controlling natural screw-worm populations	Kerrville, Tex.	Yes	13-E-4 14-E-5 15-E-1
ENT h2-17	Development of physical and mechanical methods of controlling flies and other pests of livestock	Orlando, Fla.	Yes	13-E-2 14-E-3 17-D-2 17-E

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Line		Work Locations	Summary	Area & Sub-
Project Number	Work and Line Project Titles	During Past Year	Progress	heading
ENT h2-17	Development of physical and mechanical methods of controlling flies and other pests of livestock (cont'd.)	Beltsville, Md.	Yes	13-E-2 13-F-2 14-E-3 14-F-2 17-D-2
ENT h3(R)	Cattle grub and bot fly investigations			
ENT h3-1 (R#2)	Development of new insecticides and other materials and methods for the control of grubs and bots affecting livestock	Kerrville, Tex.	Yes	13-A-7 13-B-7 14-A-7 14-B-7 14-C-1
		Corvallis, Oreg.	Yes	15-B-2 13-A-7 13-B-7 14-A-7 14-B-7
		Lincoln, Nebr.	Yes	13-B-7 14-B-7
		Stoneville, Miss.	Yes	13-B-7
ENT h4	Lice, mites, ticks, and fleas affecting man and animals investigations			
ENT h4-1 (R#2)	Development of improved insecticides and other materials and methods for the control of lice affecting livestock	Corvallis, Oreg.	Yes	13-A-9 14-A-9 14-B-9
		Kerrville, Tex.	Yes	13-B-9 14-B-9 15-A-2 15-B-3
		Stoneville, Miss.	Yes	13-B-9 14-B-9
ENT h4-3 (R#2)	Development of improved materials and methods for the control of external parasites of poultry	Kerrville, Tex. Stoneville, Miss.	Yes Yes	16-B-3 16-B-3
ENT h4-7 (R)	Development of insecticides and other methods for the control of human lice and itch mites affecting man	Orlando, Fla.	Yes	17-B-5 17-D-4,5
ENT h4-8 (R)	Development of insecticides and other methods for the area control of ticks, mites, and fleas with particular reference to protecting man	Orlando, Fla.	Yes	17-B-6 17-D-5
ENT h4-9 (R)	Development of repellents and other methods to protect man from mites, ticks, and fleas	Orlando, Fla.	Yes	17-D-5
ENT h4-10 (R)	Development of insecticides and other materials and methods for the control of ticks and sheep ked on animals	Kerrville, Tex.	Yes	13-A-10 13-B-10 13-E-6 13-F-1 14-A-10 14-B-10 14-E-6
				14-F-1 15-B-5 15-E-3

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			Summary	Area &
Number	Work and Line Project Titles	During Past Year	Progress	heading
ENT h4-11	Studies on the role of lice, mites, ticks, fleas, and other arthropods in the transmission of anaplasmosis and other diseases of livestock	Beltsville, Md.	Yes	13-G-1
		Kerrville, Tex.	Yes	14-G-1 13-G-1
		Corvallis, Oreg.	Yes	14-G-1 13-G-1 14-G-1
		Stoneville, Miss.	Yes	13-G-1 14-G-1
ENT h7	Toxicity and residue studies on insecticides and repellents in relation to the control of insects affecting livestock			
ENT h7-1 (R#2)	Investigations relating to the acute and chronic toxicity of insecticides, repellents, and other materials to livestock	Kerrville, Tex.	Yes	13-C-2 14-C-2 15-C-2
ENT h7-2 (R)	Extent of storage of insecticides in animal tissues and amount secreted in milk of dairy cattle when used for insect control	Kerrville, Tex.	Yes	13-C-1 14-C-1 15-C-1
	cause when used for insect control	Beltsville, Md.	Yes	13-C-1 14-C-1
ENT h7-4 (R)	Development of quantitative bioassay methods for analysis of insecticidal chemical residues	Kerrville, Tex.	No	
ENT hlo	Household insect investigations			
ENT hlO-1 (R)	Development of measures for the control of insects in homes	Orlando, Fla.	Yes	17-A-3 17-B-3,4 17-B-6 17-D-3
ENT jl	Identification and classification of insects			
ENT j1-1	Identification and classification of hemipterous insects	Washington, D.C.	Yes	21-B 21-C-4
ENT j1-2	Identification and classification of beetles	Washington, D.C.	Yes	21-A-3 21-B 21-C-3
ENT j1-3	Identification and classification of moths and butterflies	Washington, D.C.	Yes	21-B
ENT j1-4	Identification and classification of grass- hoppers and allied insects	Washington, D.C.	Yes	21-A-2 21-B 21-C-5
ENT j1-5	Identification and classification of two-winged flies	Washington, D.C.	Yes	21-B 21-C-2
ENT j1-6	Identification and classification of thrips	Washington, D.C.	Yes	21-B
ENT j1-7	Identification and classification of hymenopterous insects	Washington, D.C.	Yes	21-B 21-C-1
ENT j1-8	Identification and classification of mites, chiggers and ticks	Washington, D.C.	Yes	21-A-1 21-B
	(Entomological glossary - not included in a line project)	Washington, D.C.	Yes	21-A-4

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Work &			Tine Pro	oj.Inc. in
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Project		Work Locations	of	Sub-
Number	Work and Line Project Titles	During Past Year	Progres	
Number			1	nouding
ENT j2	Utilization of insect enemies in the control of insect pests and weeds			
ENT j2-4	Receipt and distribution of foreign insect enemies of insect pests and weeds	Moorestown, N.J.	Yes	21-D-1,2, 3,4,5,6,7
	00 	Berkeley, Calif.	Yes	21-D-8,9, 10
ENT 12-6	Biological control of weeds	Berkeley, Calif.	Yes	21-C-7,8
EMI JE-0	protogrear control of weeds	Rome, Italy	Yes	21-c-6
		Rabat, Morocco	Yes	21-C-5
		Rabat, Morocco	169	21-0-7
ENT j2-7 (R)	Search for and importation of foreign parasites and predators of insect pests	Paris, France	Yes	21-A-1 21-C-1,2
(11)	and produced to an experience possible and the produced possible and t	Moorestown, N.J.	Yes	21-A-1 21-C-3
		Riverside, Calif.	Yes	21-A-1 21-C-4
		Beltsville, Md.	Yes	21-C-9
ENT j2-8	Search for and importation of foreign insect	Rome, Italy	Yes	21-B-1
(R)	enemies of weeds	Buenos Aires,	Yes	21-B-1
1		Argentina		
ENT ml	Chemical investigations of products of natural origin for insect control			
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ENT ml-14	Investigation of plants as sources of insecticides, synergists, repellents, or attractants	Beltsville, Md. State College, Miss.	Yes Yes	19-A-3 19-A-3
ENT ml-15		Beltsville, Md.	Yes	19-A-1,2
	insects that might be used to upset their			
	development or reproduction or otherwise affect			
	their vital processes			
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ENT m2	Chemical investigations to develop synthetic			
	organic materials for insect control			
T	D	D-24	77.0	30 7 3
ENT m2-1	Preparation of synthetic organic compounds for	Beltsville, Md.	Yes	19-B-1
(R)	testing as insecticides and synergists	Orlando, Fla.		19 -B- 1
	D	D-24	77.0	30.70
ENT m2-4	Development of chemical formulations for insect	Beltsville, Md.	Yes	19-B-2
(R)	control	Orlando, Fla.		19-B-2
173MIT (2 7 2 2	Chemical investigations of radioactivity labeled	Poltsville Md	Yes	19- B -1
ENT m2-13	insecticides	Beltsville, Md. Orlando, Fla.		
	THECCTCIACE	oriando, Fra.	Yes	19-B-1
ENT m2-15	Preparation of synthetic organic compounds for	Beltsville, Md.	Yes	19- B -1
EHT IIIC-17	testing as insect control or eradication agents	Orlando, Fla.	Yes	19-B-1
	through effects other than death	State College, Miss.	Yes	19-B-1
	oniough circos other than acath	boase correge, Miss.	100	1 9-1-1
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Line Project Check List -- Reporting Year July 1, 1961 to June 30, 1962

Work &			Line Pro	oj.Incl.in
Line			Summary	Area &
Project	Work and Line Project Titles	Work Locations During Past Year	of Progress	Sub- heading
Number	WOLK and blue flogett flotes	During 1450 Icar	11081000	Heading
ENT m3	Analysis of pesticides, pesticide residues, and accessory materials			
ENT m3-3 (R)	Determination of residues in plant products, animal products, and soils resulting from insecticide applications	Beltsville, Md.	Yes	1-C-1,6 2-C-1 3-C-1 9-C-1 10-C-1
		Tifton, Ga.	Yes	13-C-1 1-C-2,6
				5-C-1,2,3 6-C-1,2
		Vincennes, Ind.	Yes	3-C-1 7-C-1
		Moorestown, N.J.	Yes	12-B-2
		Kerrville, Tex.	Yes Yes	13-C-1 1-C-6
		Yakima, Wash.	Ies	3-C-1 5-C-4
				7-C-2 11-C
ENT m3-5 (R)	Analysis of pesticides, accessory materials, and formulations	Beltsville, Md.	Yes	19-C
ENT m4	Chemical investigations on fumigants and aerosols for control of insect pests			
ENT m4-1 (R)	Development of formulas and dispensing equipment for insecticide aerosols	Beltsville, Md.	Yes	19 -D- 1
ENT m4-9*	Fumigants for use against the immature stages of the Japanese beetle and other soil-inhabiting insects	Moorestown, N.J.	Yes	19-10-2
ENT m9	Laboratory tests to determine the effectiveness of insect control materials			
ENT m9-1 (R)	Comparison of the toxicity of chemical materials to test insects	Beltsville, Md. Brownsville, Tex.	Yes Yes	19-E-1 19-E-1
ENT m9-3 (R)	Comparison of insecticidal materials in gas propelled aerosols and space sprays	Beltsville, Md.		19-E-3
ENT m9-4	Biological evaluation of materials for insect control through effects other than death	Beltsville, Md. Brownsville, Tex.	Yes Yes	19-E-2 19-E-2
ENT mlo	Methods for disinsectization of aircraft. (Not divided into line projects)	Beltsville, Md.	Yes	19 - F
ENT mll	Development of methods of analysis for pesticides and pesticide residues			
	* Discontinued in April 1962			

Line Project Check List Reporting Year July 1, 1961 to June 30, 1962				
Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj Summary of Progress	Incl. in Area & Sub- heading
ENT mll-2	Development of methods of analysis for insecticides, repellents, and attractants	Beltsville, Md. Orlando, Fla. Tifton, Ga. Vincennes, Ind. Moorestown, N.J. Kerrville, Tex. Yakima, Wash.	Yes Yes Yes Yes Yes Yes	19-C 19-C 19-C 19-C 19-C 19-C
Pioneering Research				
ENT P1	Insect Pathology Laboratory	Beltsville, Md.	Yes	22-A-1,2, 3,4,5,6, 7 22-B-1,2, 3,4,5 22-C-1,2 22-D-1
ENT P 2	Insect Physiology Laboratory	Beltsville, Md.	Yes	22-D-1 23-A-1 23-B-1,2, 3,4 23-C-1,2, 3 23-D-1

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